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Gill Filament Responses and Modifications During Spawning by Mouth Brooder and Substratum Brooder (Tilapiine, Pisces) Cichlids

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Abstract: Gill filaments of non-brooding and egg or wriggler brooding *Oreochromis mossambicus, O. aureus, Tilapia rendalli*, and non-brooding Taiwanese red tilapia cichlids differed in filament number, size, and shape. Filament numbers increased significantly (< 60) in female *O. mossambicus* brooding eggs or wrigglers. Filament shapes and lengths varied among species and between sexes. Egg clutch size remained constant in *O. mossambicus* regardless of brood fish size and small rather than large females brooded larger wrigglers. Filament configuration may be useful in resolving parentage of Taiwanese red tilapia or other cichlids.

INTRODUCTION

Cichlids (family Cichlidae), a dynamic speciose group of fresh and brackish water fishes, occupy myriads of habitats (Gaemers 1984, Greenwood 1991, Lowe-McConnell 1991, Nelson 1994). Their taxonomy has been subject to various interpretations (Greenwood 1987, 1991, Ponyaud and Agnese 1995, Stassny 1991).

Historically, fishes placed in the genus *Tilapia* have been divided into several genera. Trewavas (1982, 1983) divided the genera, based on type of breeding activity, into substratum brooders (genus *Tilapia*) and biparental *(Sarotherodon*) or maternal mouth brooders *(Oreochromis*) (Keenleyside 1991). Aquarists and aquaculturists still apply the term tilapia to all genera.

Tilapiine fishes have been utilized throughout the world for food. In aquaculture, hybridization efforts have produced fast growing spawners, non-reproductive unisex and sex reversed specimens or species (Schwartz 1983). This study examines the effects of brooding on the number, length, size, shape, and weight of gill filaments and arches of male and female *Oreochromis mossambicus, O. aureus, and Tilapia rendalli*, noting

especially how mouth brooding females cope with the presence of eggs or wrigglers (free swimming fry, Keenleyside 1991) in their oral chambers during the spawning season. The origin of Taiwanese red tilapia is also discussed.

METHODS

Fifty-two adult tilapiine cichlids were examined at the University of Zululand (UZ) between late January and mid-February 1988. Thirty-three specimens were supplied by the UZ Kwa Dlangezwa hatchery and 19 by the Natal Parks Board Nagel Dam hatchery (ND) near Pietermaritzburg, both in Natal Province, South Africa. They included five brooding *O. aureus* (3 male M, 2 female F, ND), 22 nonbrooding *O. mossambicus* (13M, 9F, UZ), 11 *O. mossambicus* females mouth brooding eggs (5) or wrigglers (6) (UZ), 12 substratum brooding *T. rendalli* (4M, 8F, ND), and two nonbrooding Taiwanese red tilapia of unknown parentage (1M, 1F, ND).

Specimens were measured (standard length (SL) in millimeters) and weighed (grams, g). Each of the four left gill arches were excised and weighed (GW) intact. The anterior (a) and posterior (p) hemibranch filaments were counted for each species' gill arch. Total lengths of gill filaments 10, 40, 70, 100, 110, 130, 160, and/or 190 were measured using a Zeiss microscope (set at 10 × where 120 ocular Wild micrometer units = one milli-

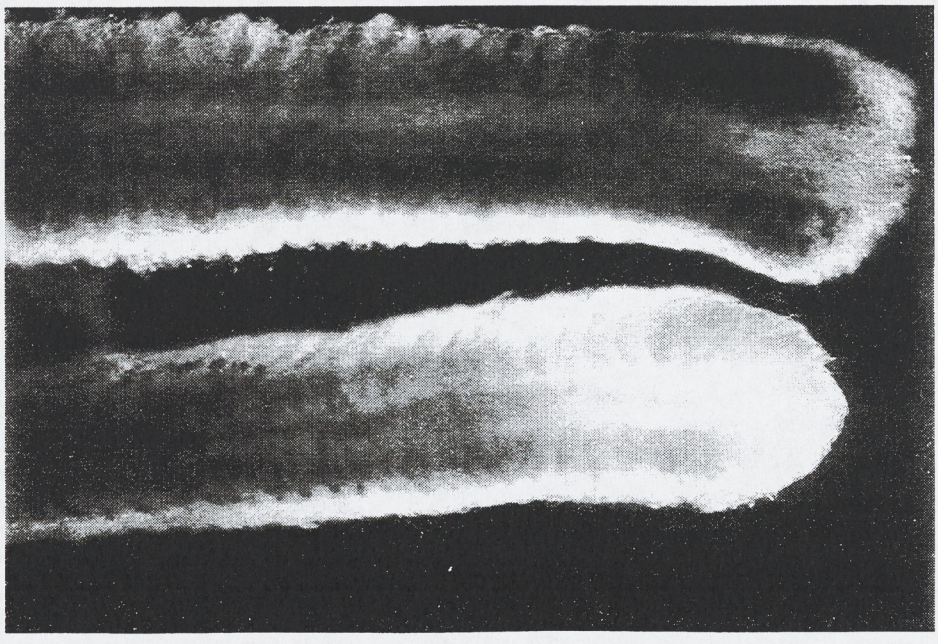

Fig.1. Filament shapes *O. mosambicus* (1B) and *O.aureus* (1A), note secondary lamellae crossing each filament.

meter). Gill filament 110's length and measurement was included as it was the longest filament of each arch, regardless of brooding condition. Filament widths were measured of each arch's selected a and p filaments at their base (B), middle (M), and tip (T) to note shape (Fig. 1a, b) or "area"-length changes (Figs 2, 3). Lengths and heights of several secondary gill filament lamellae (Fig. 1a, b) were measured but not used in "area" calculations.

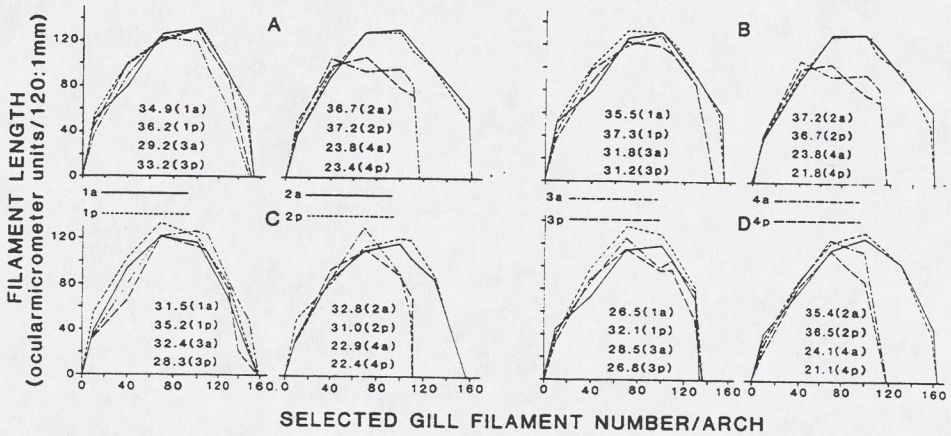

Fig. 2. Mean lengths of male (A), nonbrooding female (B), females brooding eggs (C) or wrigglers (D) *O. mossambicus*. (a) anterior (p) posterial filaments. Numbers indicate area(s) under each arch a or p curve.

"Areas" under a and p filament length curve graphs were determined using a Calcomp 9 500 digitizer and Autocad program (Figs. 2, 3). Linear analyses of log y = a + b log x defined the relationships: SL/body weight (BW), total arch weight/BW, total arch filament number (N)/BW, total a and p (N)/SL, and total a filament length/total p filament length. ANOVAs tested, by species, sex, and brooding condition: a and p(N) differences, gill arch weight (GW)/N, and brooding female *O. mossambicus* (G W X N)/SL and (G W X N)/BW relationships. Employing standard mathematical designations, significant results > 0.5 were denoted by an *, > 0.01 highly significant **, and > 0.000 1 very highly significant ***. Each brooding female *O. mossambicus* egg mass was weighed and the eggs counted and measured (length x width, mm). The total mass of wrigglers occupying the oral cavity of a *O. mossambicus* was weighed and the wrigglers counted and measured (total length, mm).

Following study, all specimens were preserved with their right holobranchs and filaments intact in 10% formalin and later transferred to 70% isopropyl alcohol. Specimens were then deposited at the University of North Carolina, Institute of Marine

Sciences, Morehead City, North Carolina, USA as: *O. mossambicus* UNC 17126 (includes "normal males and females", brooding females and their eggs or wrigglers, and Taiwanese red tilapia), *O. aureus* UNC 17127, and *T. rendalli* UNC 17128.

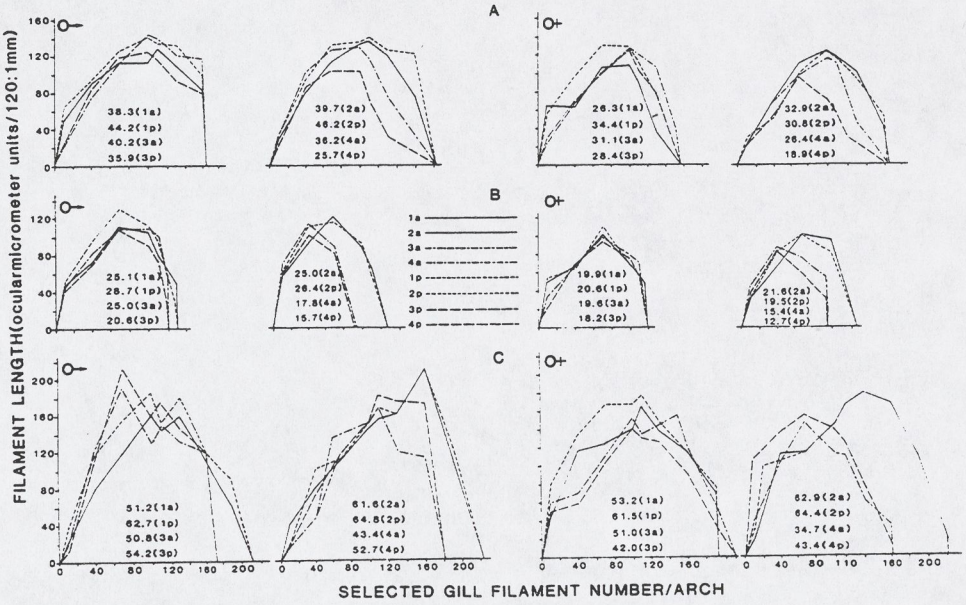

Fig. 3. Same depictions as in Fig. 2. for *O. aureus* (A), *T. rendalli* (B), and Taiwanese red tilapia (C).

RESULTS

General: Gill filament number (N) and weight (W) of male (M) and female (F) Taiwanese red tilapia were the highest and heaviest of all species studied (Total Male (N) = 1564, 5.98 g; Female (N)=1578, 4.70 g, Table 1, Fig. 4). Filaments 40 through 160 were broadest at their bases and middles, while their tips were narrow and pointed (Fig. 1A). Male and female *O. aureus* possessed the second highest gill filament N and W (M(N) x = 1 279, F(N) x = 1 235, M(W) x = 2.20, F(W) x = 1.90, Tab. 1) even though N and W differences were statistically non-significant (M vs. F(N) f = 0.32, M vs. F(W) f = 0.53). Female *O. aureus* gill filament N totals exceeded those of female *O. mossambicus* but both M and F *O. aureus* filament ranges fell within those noted for *O. mossambicus* (Tab. 1, Fig. 4). Non-brooding male and female *O. mossambicus* gill filament N totals were statistically highly different; males possessed more and heavier gill filaments and archs than non-brooding females (M(N) f = 5.57**, F(N) f = 6.28**, Tab. 1). Female *O. mossambicus* brooding eggs or wrigglers possessed statistically more gill filaments (66 and 61 respectively) than non-brooding females (Tab. 1). Brooding male and female

T. rendalli total gill filament N and W differences were not significantly different (M vs. F(N) f = 1.97, M or F(E) f = 0.85) even though female filament ranges exceeded those of males (Tab. 1, Fig. 4). Both male and female *T. rendalli* possessed, of the species, examined the lowest number of gill filaments (Tab. 1, Fig. 4).

Table 1. Number, sex, SL, body weights of various brooding on non-brooding cichlids studied and numbers and ranges of gill filaments, gill arch total weights, and eggs and wrigglers measured. See Figs. 2 and 3 for length of selected gill filaments/arch/species/brooding condition.

			SL (mm)		Body Weight (g)		Total filaments (4 arches)			Gill Arch Total Weight		Eggs or Wrigglers			
Species/condition	N	Sex	$\bar{x}$	Range	$\bar{x}$	Range	N counted	$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range	x	Range (g)
O. mossambicus	13	M	168	142-240	157	99-420	15150	1109	1029-1402	2.20	1.43-5.33				
	9	F	145	123-165	101	66-138	9836	1093	1012-1192	1.50	0.85-2.11				
O. mossambicus with eggs	5	F	157	146-164	118	101-140	5796	1159	1018-1234	1.73	1.36-2.22	250	10-424*	2.28	0.2-4.2
with wrigglers	6	F	161	150-170	122	106-140	6926	1154	1106-1230	1.78	1.20-2.06	354	159-410**	3.56	2.3-6.0
O. aureus	3	M	160	157-206	191	128-282	3828	1279	1180-1382	2.78	1.77-4.43				
	2	F	160	144-175	129	93-165	2470	1235	1206-1264	1.90	1.43-2.37				
T. rendalli	4	M	173	145-180	192	124-226	3854	964	948-976	1.68	1.01-1.81				
	8	F	148	115-199	117	53-240	7228	904	838-1026	0.97	0.41-2.12				
Taiwanese red tilapia	1	M	212	—	323	—	1564	1564	—	5.98	—				
	1	F	227	—	401	—	1578	1578	—	4.70	—				
Total	52						58230								

* egg length x width (mm) x = 2.38 x 2.61, range 2.15-2.57 x 2.45-3.04
** wrigglers length (mm) x = 8.24 range 7.13-8.74

Each species' gill arch's a and p total number of filaments was the same/arch regardless brooding condition. Gill arch 2 possessed the most filaments followed by either arch 1 or 3 (depending on species or brooding condition) and arch 4 (Figs 2, 3). Each species' p filaments were usually longer than its a filaments (Figs 2, 3). This was especially true for male *O. aureus*, Taiwanese red tilapia, arches 1,2, and 3 of *T. rendalli*, and arches 1 and 2 of *O. mossambicus*. Female arches 1 and 2 a and p filaments were longer than those of arches 3 or 4 in "normal" *O. mossambicus*, *O. aureus*, and *T. rendalli*, whereas *O. mossambicus* arches 1 and 3 filaments were longer in females brooding eggs versus arches 1 and 2 in females brooding wrigglers (Fig. 2).

O. mossambicus: *O. mossambicus* specimens, other than the two Taiwanese red tilapia, possessed more total gill filaments than did *O. aureus* or *T. rendalli* (M filament (N) x = 1 109, range 1 029-1 402, F(N) x = 1 093, range 1 012-1 192, Tab. 1, Fig. 4). Lengths and shapes of selected a filaments of males, non-brooding females, and brood-

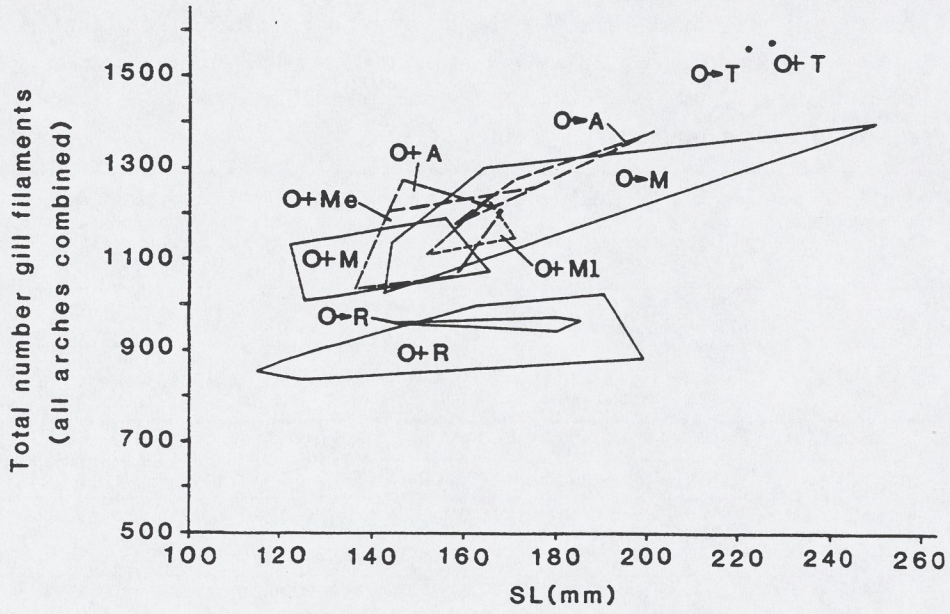

Fig. 4. Total gill filament variation, by size of male and female *O.mossambicus* (M) and females brooding eggs (Me) or wrigglers (M1), *O. aureus* (A), *T. rendalli* (R), and Taiwanese red tilapia (T).

ing females with eggs or wrigglers were usually longer in males than females, especially in filaments 100 and 110 (Fig. 2). The selected filaments, regardless of sex or brooding condition, also had wider bases and middles than tips (Fig. 5). Most importantly, the number (and weight) of gill filaments increased in females brooding eggs by 66 or wrigglers by 61 (Fig. 6). Filament number increases were greatest in females brooding eggs, on arch 4 (12.5%) followed by arch 1 (9.6 %), arch 3 (5.5 %), and arch 2 (5.2 %). In females brooding wrigglers, arch 4 (15.6%) reflected the greatest filament increase followed by arch 2 (7.4 %), arch 1 (5.4 %), and no increase in arch 3. Note how increased shape of the filaments was verified by a corresponding decrease in the total "area" under each arch in female brooding eggs or wrigglers (Fig. 2).

O. mossambicus SL/W relationships were highly correlated (M, r = 0.9322; F, r = 0.915 8), in females brooding eggs r = 0.940 5 or wrigglers r = 0.9421. Male, non-brooding female, and brooding female gill filament lengths varied by hemibranch and brooding conditions, but length differences were non-significant (M vs. F, f = 0.0016, F vs. F with eggs f = 0.75, and F with wrigglers f = 1.00). Important very high significant relationships were gill W x gill N for females brooding eggs f = 125.0*** or wrigglers f = 564.97***, regardless of SL or W of fish (Fig. 5). Likewise highly significant

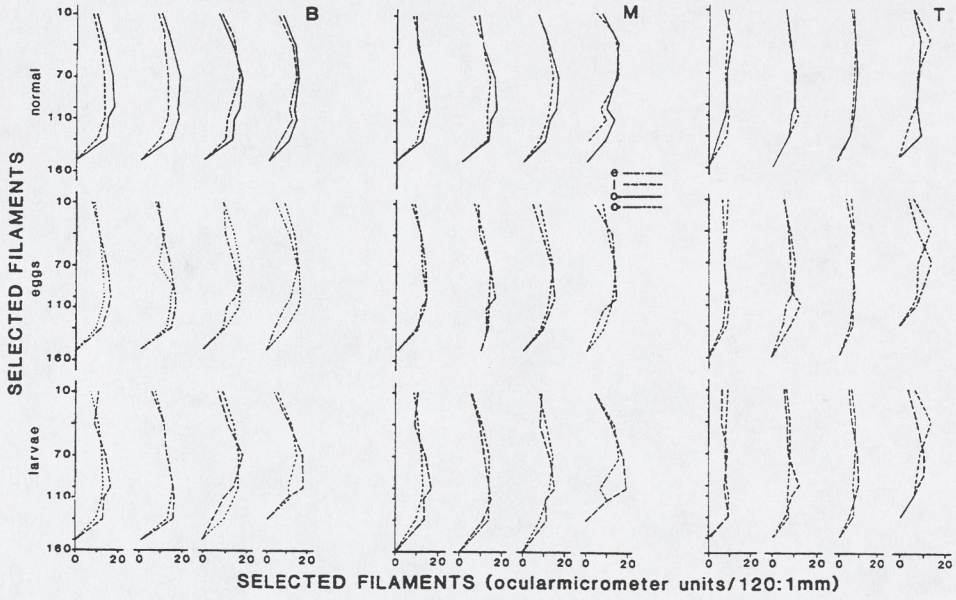

Fig. 5. Base (B), Middle (M), and Tip (T) shapes of *O. mossambicus* gill filaments/arches 1-4 (left to right in each depiction) exhibited by males and nonbrooding females (top row), and nonbrooding (-) and brooding (--) females with eggs (middle row) or wrigglers (lower row).

G W X N/SL relationships existed for females brooding eggs f = 33.53** or wrigglers f = 46.82** and G W X N/BW (females with eggs f = 6.66**, wrigglers f = 10.70**). The most eggs brooded by study females was 424 yet the mean number of eggs, measuring 2.38 × 2.61 mm, remained about 250, regardless of female SL (Tab. 1). About 354 wrigglers (range 159-410) measuring x = 8.24 mm (range 7.13-8.74 mm TL) usually comprised a brood. The smallest wrigglers were carried by the largest females (log y = 1.651 - 0.336 9 log x, r = -0.222 7, N = 10).

O. aureus: O. aureus males and brooding females possessed heavier and more gill filaments than *O. mossambicus* (Tab. 1, Fig. 4). Filament "area"-lengths were very highly significant between sexes, f = 374.80***. Filament a and p numbers were highly significant f = 12.41**, with the longest filaments on arch 2. Gill arches 1 and 2 contained the most filaments and arch 4 the least and shortest filaments. Male *O. aureus* filaments were shortest and highest on arch 4 (Fig. 3) and were longest between filaments 70 and 160; female filaments were longest on arches 1 and 2 (Fig. 3). *O. aureus* was unique in that filaments 70-160 of both sexes became broader at their midpoint and tips, giving the filament a reversed deltoid shape (Fig. 1b).

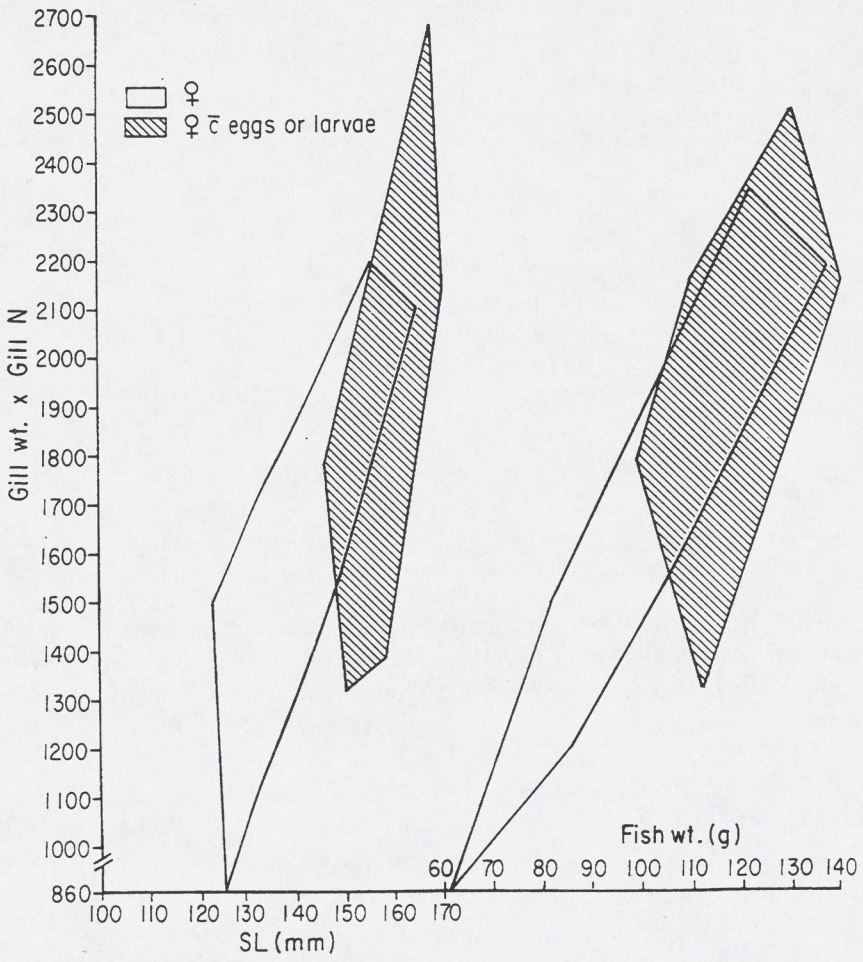

Fig. 6. Ranges of gill arch weights x gill N/SL or BW in female *O.mossambicus* brooding eggs or wrigglers.

T. rendalli: A substratum brooder, *T. rendalli* had the smallest filaments and lightest gill arches (Tab. 1, Fig. 3). The p filament lengths were longer than a lengths in both sexes (Fig. 3). Filament lengths were significantly different between sexes ($f = 5.30^*$) and arch ($f = 4.30^*$), but not between a and p lengths. Gill arch 2 and 3 filaments were broadest at their base, and their tips were pointed (Fig. 1a). Females possessed heavier filament arches than males (Tab. 1).

Taiwanese red tilapia: Filament lengths of the two Taiwanese red tilapias varied widely (Fig. 3). No statistical differences existed between the sexes or a and p numbers even though arch weights were highly significantly different ($f = 15.73^{**}$). Arches 1

and 2 had longer filaments in both sexes. On all arches filaments 100-130 were the longest. Arch 4 was heavy and wide (Tab. 1). Filament shape varied from narrow at the base to wide at midpoint to pointed at the tip.

DISCUSSION

Fish gills are multi-functional organs that serve to transport oxygen and other ions to or from the fish (Laurent and Perry 1991). Gray (1954) was one of the first to study fish (marine) gill arches. During the subsequent 20 years, Hughes (1966, 1972, 1984), Hughes and Morgan (1993), Palzenberger and Pohla (1989) described the development and functions of fish gills, and ways to calculate their areas, based on total filament length. Others related gill areas to fish size (Muir 1989; Santos et al. 1984) or body weight (Ojha and Singh 1987).

Among cichlids Kuwamura et al. (1989) noted the number of young in mouth brooding *Sarotherodon markieri* decreased with growth of their fry and brood size was correlated with body weight of the female. Barel et al. (1991) found that total gill filament number increased with fish size in *Haplochromis hiatus* and *H. iris* and especially in *H. iris* inhabiting low oxygen content habitats. Hoogerhund et al. (1983) commented that *O. niloticus* had 2 750 filaments, and the gill area increase in fish inhabiting hypoxic conditions may be due to food differences and feeding. Fernandes and Rentin (1986) found that increased gill area was an adaptation to hypoxia as a way of increasing the oxygen extraction from the surrounding water, and that filament N and length increased with body weight. Balshine-Earn (1995) noted the high costs of weight loss in male and female brooding *Sarotherodon galilaeus*. Keenleyside (1991) commented on mouthing or churning (rolling the eggs or wrigglers) as a way to keep eggs or wrigglers clean and aerated, as the bottom of the mouth cavity was an area of low oxygen water. Lowe-McConnell (1959) noted that the number of gill rakers increased as one progressed from bi-parental mouth brooders to maternal mouth brooder tilapias.

In this study, *O. mossambicus* male and female filament numbers were significantly different and between non-brooding and egg or wriggler brooding individuals. Their filament bases were usually ovate, except near the tips of arches 3 and 4 filaments, where they were deltoidal. Differences noted in brooders were the result of filament N and W increases, not length. This permitted increased water flow over the gills and eggs or wrigglers. *O. aureus* had the most variable shaped gill filaments studied, varying from deltoid to cordate. Filament tips were trunicated (Fig. 1B).

O. aureus is known to hybridize with *O. mossambicus* (N'gokaka 1983, Wohlfarth et al. 1990) yet the low filament numbers observed rule out *O. aureus* a parent of the

Taiwanese red tilapia. *T. rendalli* possessed the lowest number gill filaments and arch weights. No gill filament changes occurred in nonbrooding or brooding *T. rendalli.*

Taiwanese red tilapia: N'gokaka (1983) and Wohlfarth (1990) reviewed the inhabitance of red color and its hybrid origin in tilapias. Possessing a high gill filament number, Taiwanese red tilapia may be a hybrid of *O. mossambicus x O. niloticus*. The lineage of Taiwanese red tilapia is complicated (N'gokaka 1993, Wohlfarth et al. 1990) and resolution may be impossible for there is a dearth of information on gill filament number and size within tilapiine fishes.

CONCLUSIONS

Gill filament shapes, sizes, numbers, and structure change during mouth brooding. Brooding females adapt morphologically and physiologically by adding more and heavier gill filaments. These features may be useful in determining the parentage of hybrids.

Acknowledgments

This paper is dedicated to my longtime friend and colleague Dr. Ota Oliva, who was the Czech Republic's most outstanding ichthyologist, herpetologist, scientist, teacher, and scholar. His loss to the scientific community and the country is great and will be long remembered. Dr. W. Barham, University of Zululand, and Tom Pike, Natal Parks Board, provided the study cichlids. Dr. Y. Visser provided work space in the Department of Zoology (UZ). The graciousness of the University of Zululand administration will be long remembered for their providing facilities and other amenities. Dr. R. J. Goldstein, Raleigh, North Carolina, reviewed the manuscript. G. Safrit and M. Bylerly (IMS) assisted with the statistical analyses, J. McNinch the "area" analyses, R. Barnes produced the figures, and L. White the text.

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In review

Genetic evidence for allopatric and sympatric differentiation among morphs of a Lake Malawi cichlid fish

Abstract: Four sympatric color morphs of the cichlid fish Pseudotropheus zebra were found to have significant differences in allelic frequencies at 4 polymorphic loci and to form two distinct mating groups. Northern and southern populations of the common Barred morph were also found to have significant differences in allelic frequencies at 5 loci. The data support the view that rapid cichlid differentiation can be caused both by extrinsic geographic isolation of populations as well as by intrinsic behavioral factors which could lead to sympatric speciation.

The species flocks of cichlid fishes within the Great Lakes of East Africa are the most spectacular examples of speciation and of adaptive radiation within a single vertebrate family. Each of the three lakes, Malawi, Tanganyika and Victoria, contains more species of fish than any other lake in the world. Lake Malawi has about 300 described species of fish, 90% of which are cichlids and between 100-200 species still to be described or discovered (1). Other outstanding examples of fish speciation in lacustrine environments--the 18 endemic cottids of Lake Baikal (2), the 18 cyprinids of Lake Lanao (3) and the 14 endemic cyprinodont species of the genus Orestias of Lake Titicaca (4)--show but 1/20th of the species diversity of cichlids in Lake Malawi. Thus, these cichlid fishes, exhibiting the most outstanding case of explosive evolution in modern vertebrates, offer extraordinary opportunities to study the factors that determine the evolution and speciation processes of a highly complex community of closely related animals.

An important genetic study by Sage and Selander (5) has, however, cast doubt as to whether or not the cichlid flocks are as species rich as previously reported (6). They discovered that cichlid morphs from lakes in the Cuatro Cienegas region of Mexico, which have been treated as an endemic species flock (7), could not be distinguished electrophoretically and were thus a single polymorphic species. These results suggest that much of the trophic radiation in the African cichlids also might have been achieved through polymorphism. Sage and Selander (5) suggest that much of the variation observed in the African cichlid flocks might not

represent speciation, and emphasize the importance of a genetic approach to the problem of whether adaptive radiation or speciation came first. Their challenge of the classical view (8), that trophic radiation occurs after speciation, has led others (9), to question whether phenetically defined cichlid species are, in fact, conspecific.

The conflicting views concerning the sequence of adaptive radiation and speciation and the validity of many of these cichlid species is, in addition, interwoven into the controversy over allopatric vs sympatric speciation in this family. Greenwood (8) suggests that complete isolation of cichlids in separate basins in Lake Victoria led to allopatric differentiation between species. Fryer (10) concludes that the diversity of cichlids in Lake Malawi has arisen through microallopatric speciation. This view corresponds with the hypothesis of others (11) who suggest that populations of ancestral Lake Tanganyika cichlid species were geographically isolated and hence speciated allopatrically. Mayr (12) cites Fryer's work in dismissing the possiblity that sympatric speciation might, in part, explain the number of cichlids in these African lakes. However, the allopatric hypothesis has never been accepted as an exclusive one by biologists concerned with cichlids (13,14,15) or by those who find the evidence in support of sympatric speciation highly suggestive (16). McKaye (14) suggests from field evidence that "it seems likely that some cichlid species have arisen because of ethological differentiation of morphs in mating and habitat preferences without a geographical barrier."

Recent ecological and behavioral studies have suggested that 4 common color morphs of the Malawi cichlid, Pseudotropheus zebra, show complete assortative mating, and form two distinct mating groups (15). The authors of these studies conclude, in contrast to Sage and Selander (5), that the morphs must be considered segregated gene pools and that they diverged sympatrically. If true, this leads to the question of how assortative mating by sympatric morphs evolved.

These multiple controversies, the importance of the questions raised, and the continual discoveries of new cichlid taxa in these African lakes (17) led us to test the Sage and Selander hypothesis for African cichlids. We examined electromorph data collected for color morphs of P. zebra from Nkhata Bay, Lake Malawi. These morphs are described in detail elsewhere (7,15) and will be referred to here as Barred (BB=blue and black vertical bars), Orange-Black (OB=orange with black spots), Blue (B=homogeneous blue) and White (W=predominantly white) (Fig. 1). We examined all four morphs from Nkhata Bay in the north central portion of Lake Malawi. In addition, to determine allopatric differentiation, we examined the common Barred morphs from three other sites in different geographic regions in the lake: two sites from the southern region, Mumbo Island and Domwe Island, and one site further north at Chilumba. The Blue and White morphs are known to occur only in the northern regions of the lake whereas Barred and Orange-Black morphs are found throughout the lake (7,15).

Protein electromorphs were studied with standard horizontal starch-gel electrophoresis and histochemical staining procedures (18). Liver, muscle, heart and eyes of adult fish were removed in the field and stored frozen in 0.01 Tris-HCl, pH 7.0 (containing 5 mM dithiothreitol and 0.5% polyvinylpyrollidone-360) at -196° C in

liquid nitrogen for 4-6 weeks. The samples were transferred to a freezer (-60° C) on return to the laboratory and remained there 3-8 months until analysis (19). Before beginning data collection, we surveyed 24 loci (20) to identify polymorphic systems. We examined intensively in our analysis 7 loci which we determined to be variable in our survey.

There are two main results. First, the allelic frequencies of 4 polymorphic loci support the earlier view (15) that assortative mating and two gene pools are present within this species from Nkhata Bay (Table 1). For Pep the frequency of the b allele was 78% for Barred and Orange-Black morphs and 1% for the Blue and White morphs. The results of Sdh, Pgi and Est-3 also partition by these breeding groups (Table 1). The genetic data, thus, are consistent with the ecological evidence that Barred and Orange-Black mate assortatively with each other and that Blue and White do also. However, although the data strongly suggest separate gene pools occur, we cannot conclusively state on the basis of genetic evidence alone that these two sets of morphs are distinct species.

Second, there are significant differences between the allelic frequencies of both macro- and micro-allopatric Barred populations (Table 2). For Pep, 88% and 68% of the alleles of the southern populations, Mumbo and Domwe, respectively, are a, whereas 80% and 79% from the northern Chilumba and Nkhata Bay populations, respectively, are b (G test, $p < .01$). There are also statistically different allelic frequencies between the northern and southern populations for Sdh, Got and Est-1 in all cases (G test, $p < .01$). Interestingly, for Est-3 there are distinct differences between Domwe Island and Mumbo Island populations. The frequency of the

b allele is 27% for the Mumbo population and 0% for Domwe; whereas the frequency of the d allele is 32% for Domwe and 0% for Mumbo (Table 2). Except for this, these two southern populations are indistinguishable from each other at the loci examined. They, however, do differ consistently from the two northern populations, which in turn are similar to each other.

The results, thus, bear on the 2 issues raised initially, the species richness of African cichlid flocks and the mechanism of speciation. Unlike Sage and Selander's (5) initial study on Mexican cichlid morphotypes, the morphs of P. zebra do form separate gene pools and might be considered two separate species, as has been suggested by others (15). With regard to the process of differentiation among cichlids, these data demonstrate macro-allopatric genetic differences between the northern and southern populations of this variable species. These populations, which are rock dwelling, are separated by long sandy beaches in the central region of Lake Malawi. Over 95% of the shoreline between Nkhata Bay and the Nankumba peninsula, where Domwe and Mumbo Islands are located, is sand and marsh (21). Between the northern populations at Chilumba and Nkhata Bay there are long rocky stretches which should facilitate migration and gene flow. Over 35% of the shoreline between these two populations is solid rock substrate. Furthermore, the difference in the Est-3 locus for Domwe and Mumbo Islands may indicate relatively recent isolation and thus lend support to Fryer's micro-allopatric speciation model.

How, then, can the evolution of assortative mating by sympatric cichlid morphs be explained in the context of allopatric speciation processes alone? Conceivably the Blue and White morphs were at one

time geographically isolated from the Barred and Orange-Black morphs. They began mating together in that isolation and then subsequently became sympatric with the other morphs. This allopatric hypothesis requires either the extinction of the widespread Barred and Orange-Black morphs in an area where the Blue and White morphs occurred or the colonization of a new area by the Blue or White morphs where the ubiquitous Barred and Orange-Black morphs were not present.

The alternate, sympatric model of Maynard-Smith (22) does not require such past geographical isolation of populations. His model instead requires that morphs assortatively mate and choose somewhat different habitats. Futuyma and Mayer (23) claim that selection of different "niches" by sympatric morphs of a single species has not been demonstrated. They conclude, therefore, that Maynard Smith's model is unlikely to explain speciation. But such niche differentiation among cichlid morphs has been observed (14,15, 24), and suggests that Maynard Smith's model is plausible. Furthermore, if intrinsic behavioral factors are important in speciation as predicted by Maynard-Smith, we should expect that species splitting would be more rapid than if extrinsic geographical barriers are required to occur and breakdown in order to isolate and reassociate populations.

Maynard Smith's hypothesis of sympatric speciation, therefore, cannot yet be rejected for the family Cichlidae. The elements necessary to make Maynard Smith's model a likely possibility are documented for several cichlid genera: 1) a high rate of speciation, 2) morphological polymorphisms, 3) gradations of assortative mating, and 4) differential habitat selection by morphs. Cichlids are highly polymorphic in tooth form and in coloration pattern (6,9). Numerous authors have concluded that coloration is important in

in species recognition in the family Cichlidae (5,9,14). In the Cuatro Cienegas cichlid no assortative mating by morphotype is evident (6). However, in Lake Jiloa, Nicaragua, two color morphs, gray and gold, of Cichlasoma citrinellum tend to mate assortatively and morphs select different habitats in which to breed. This situation could be a stage in the formation of a deep water gold species and a shallow water gray one (14). In Lake Malawi, Marsh et al. (25) have concluded recently that what was previously thought to be a single Lake Malawi species, Petrotilapia tridentiger, can be divided into at least 3 distinct species on the basis of assortative mating by color pattern and differential bathymetric distribution by those color patterns. Yet the genetic differences between these forms (26) are not as great as those we observed here in the Pseudotropheus zebra morphs (Table 1).

This study is the first to document genetic differences among cichlid color morphs which suggest that morphs may be genetically isolated or, if not yet completely isolated, are mating assortatively. In addition this study demonstrates that both macro- and micro-allopatric differences between populations are clearly present. A subset of the data, the differences between northern and southern populations, lends support to the concept of macro-allopatric differentiation of populations and possible eventual speciation (8,12). Another subset of the data, the differences between the populations of the southern islands of Mumbo and Domwe, suggest that micro-allopatric differentiation (10) is also occurring. In addition, the final subset of the data, the differences between assortatively mating morphs within the population at Nkhata Bay, suggest that sympatric differentiation (22) can also occur.

Clearly the genetic data support the suggestion from field data that the hypotheses of allopatric and sympatric speciation are not necessarily mutually exclusive. Both sympatric and allopatric speciation are most likely occurring simultaneously, and intrinsic behavioral factors may be important in explaining the rapid rate of cichlid speciation. More comparative data, however, on the frequency of polymorphisms, assortative mating, habitat selection by morphs, and survivorship of heteromorphic offspring are required before we can assess the relative importance of sympatric speciation compared to that of the more predominant allopatric speciation mode. The evidence suggests, in the case of the cichlids of the Rift Valley Lakes of Africa, that there are more, rather than fewer, species present than are now described. Furthermore, they have arisen and are continuing to arise through a combination of sympatric and both macro- and micro-allopatric processes of differentiation and speciation (27).

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19. The fish from Chilumba and half of the Blue and White morphs were collected by Mr. Eric Fleet and shipped live and kept in aquaria. The tissues from these fish were quick frozen in liquid nitrogen and then used immediately.

20. We found only one electromorph for the following enzymes: CK, GP-1, GP-3, 6PGD, G6PD, mGPdh, HK, Idh, Est-4, ME, Mdh, Ldh, Adh, Pep-2, Pep-3, PGM-1, TO. We found more than one electromorph for Est-1, Est-3, Pep-1, Pgi-1, Pgi-2, Sdh, Got.

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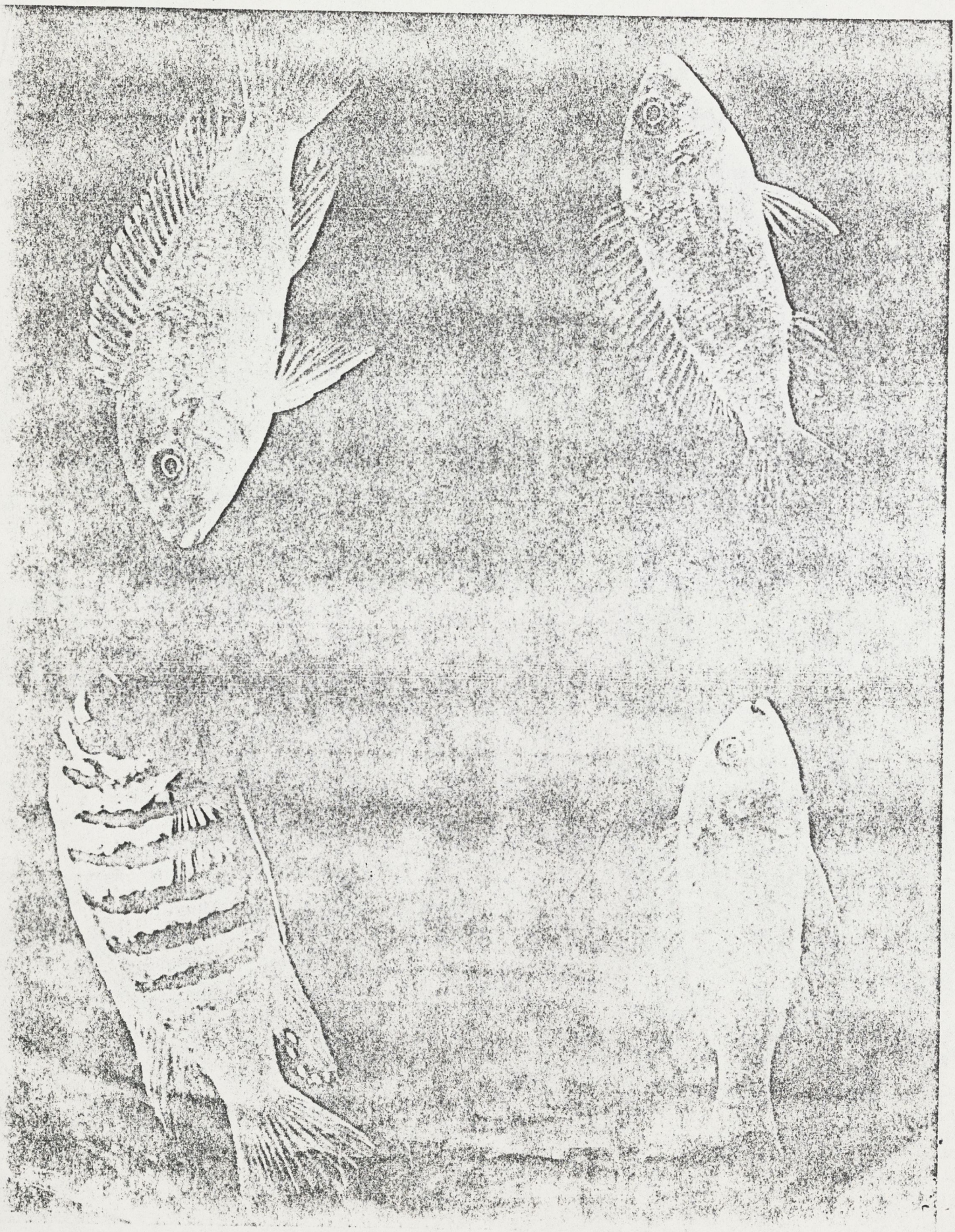
Table 1. Electromorph frequencies of color morphs of P. zebra at Nkhata Bay, Lake Malawi, Africa. * $p<.05$, ** $p<.01$ (G test)

Morph	N (alleles)	Allele a	b	c	d	e	Heterogeneity G (d.f.)
			Pep				
BB	34	0.21	0.79				
OB	34	0.23	0.77				111.6 (3)**
B	44	1.00					
W	34	0.97	0.03				
			Sdh				
BB	34	0.30	0.70				
OB	34	0.27	0.73				18.9 (3)**
B	46	0.04	0.96				
W	36	0.03	0.97				
			Pgi-1				
BB	34	0.06	0.82		0.12		
OB	32		0.94		0.06		15.8 (6)*
B	48		1.00				
W	36		1.00				
			Pst-3				
BB	34	0.23	0.71	0.06			
OB	34	0.06	0.71	0.06	0.18		110.6 (12)**
B	44	0.04	0.14	0.50	0.42		
W	36		0.06	0.36	0.47	0.11	

Table 2. Electromorph frequencies of BB morphs at various localities in Lake Malawi, Africa. * $p < .05$, ** $p < .01$ (G test)

Location	N (alleles)	Allele a	b	c	d	Heterogeneity G (d.f.)
			Pep			
Mumbo I.	34	0.88	0.12			
Domwe I.	34	0.68	0.32			58.5 (3)**
Nkhata Bay	34	0.21	0.79			
Chilumba	54	0.20	0.80			
			Sdh			
Mumbo I.	34		1.00			
Domwe I.	34		1.00			24.4 (3)**
Nkhata Bay	34	0.29	0.71			
Chilumba	50	0.12	0.88			
			Got			
Mumbo I.	34	0.41	0.59			
Domwe I.	34	0.29	0.71			18.0 (3)**
Nkhata Bay	34	0.03	0.97			
Chilumba	54	0.18	0.82			
			Est-1			
Mumbo I.	34	1.00				
Domwe I.	32	1.00				23.2 (6)**
Nkhata Bay	34	0.85	0.15			
Chilumba	54	0.76	0.22	0.02		
			Est-3			
Mumbo I.	34	0.18	0.27	0.56		
Domwe I.	32	0.12		0.56	0.32	50.6 (9)**
Nkhata Bay	34		0.23	0.71	0.06	
Chilumba	54	0.02	0.06	0.81	0.11	

Fig. 1. Color morphs of Pseudotropheus zebra: Barred, Blue, White and Orange-Black. Photos by K. R. McKaye and W. Sacco.



IN REVIEW

Genetic analysis of a sympatric sibling species complex of *Petrotilapia* Trewavas (Cichlidae, Lake Malawi)

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Running title: Genetic analysis of *Petrotilapia*

Abstract. The protein electromorphs of three taxa of Petrotilapia were examined with starch-gel electrophoresis. These 3 taxa, referred to in earlier work as sibling species, were found to have no alternate fixed alleles at any of the 25 loci examined. However, heterogeneous gene frequencies were found at 7 polymorphic loci. These results suggest that these taxa are isolated "sibling" species which recently diverged or they are "incipient" species with minimal gene flow between morphs. The genetic and field data lend support to the hypothesis that sympatric splitting of morphs could be important in the explosive radiation of the Cichlidae.

KEY WORDS:--Evolution, speciation, polymorphism, genetics, fish, electrophoresis, mate selection, cichlid

CONTENTS

INTRODUCTION

The species flocks of cichlid fishes within the Rift Valley Lakes of Africa represent a classic case of explosive speciation (Fryer & Iles, 1972; Greenwood, 1974). Lake Malawi, for example, has almost 300 described cichlid species (Marsh et al., 1981) with many more awaiting description and discovery (D. Eccles, D. Lewis, A. Marsh, M. Oliver, A. Ribbink personal communication; K. McKaye personal observation). Despite the overwhelming evidence of diversity among cichlids of all three of the large great lakes of Africa, a genetic study of a polymorphic Mexican cichlid (Sage & Selander, 1975) has been used to cast doubt upon whether or not the African cichlid flocks are really as species rich as reported (Graves & Rosenblatt, 1980; Kirkpatrick & Selander, 1979). Sage & Selander (1975) found that cichlid morphs from lakes in Cuatro Cienegas region of Mexico, which have been treated as an endemic species flock (Kornfield & Koehn, 1975), could not be distinguished electrophoretically. Their results suggest that the morphs represent a single polymorphic species. Sage & Selander (1975), in addition, propose that much of the variation observed in the African cichlid flocks may represent polymorphism rather than speciation, and emphasize the importance of genetic data to resolve such a question.

Recent field studies of Lake Malawi cichlids have suggested, in contrast, that species which were previously thought to be polymorphic may begroups of sibling species (Holzberg, 1978; Schroder, 1980; Marsh et al., 1981). These studies on two of the Lake Malawi cichlids, Pseudotropheus zebra and Petrotilapia tridentiger, suggest that sympatric morphs separate into different gene pools on the basis of assortative mating by color morphs. Petrotilapia

tridentiger, which has been considered a single polymorphic species (Fryer, 1959), was divided into 3 sibling species, referred to by Marsh et al. (1981) as Big Blue (BB), Orange Cheek (OC) and Orange Lappet (OL). Field observations of noninterbreeding among color morphs, underlie the suggestion by Marsh et al. (1981) that the 3 are distinct, sibling species. This conclusion is reached inspite of the taxa being indistinguishable on the basis of morphometrics.

Both the controversy over the actual diversity of cichlid fishes in the great lakes and the importance of assortative mating to theories concerning the speciation process (Maynard Smith, 1966; McKaye, 1978; 1980) led us to examine electrophoretically enzyme polymorphisms within these three "sibling" species of P. tridentiger. The two questions on which we focused were: 1) Are different alternate alleles fixed at any testable locus for the 3 taxa? 2) Are gene frequencies homogeneous or heterogeneous among taxa? Reproductive isolation between species would be demonstrated unambiguously if alternate alleles are fixed at an isozyme locus (Ayala & Powell, 1972). Heterogeneous gene frequencies among color morphs would suggest that these taxa are not interbreeding randomly. Homogeneous frequencies among color morphs would suggest either: 1) a high degree of interbreeding among the groups, or 2) parallel variation at the loci tested.

METHODS

Protein electromorphs for the three taxa were examined with standard horizontal starch-gel electrophoresis and histochemical staining procedures (Selander et al., 1971). The fish used were captured by being herded into a fine mesh net in Monkey Bay

at the southern end of Lake Malawi. Alan Marsh (of the J. L. B. Smith Institute of Ichthyology, Grahamstown, South Africa) caught and identified all specimens for us. Sample sizes were: 17 males and 2 females of Orange Cheek, 19 males and 1 female of Orange Lappet, and 13 males and 9 females of Big Blue.

We removed liver, muscle, heart and eyes of adult fish in the field and stored them frozen in 0.01 Tris-HCl pH 7.0 (containing 5 mM ditheiothretiol and 0.5% polyvinylpyrollidone-360) at -196° C in liquid nitrogen for 4-6 weeks. The samples were transferred to a freezer (-60° C) on return to the laboratory in the U.S.A. and remained there 8 months until analysis.

RESULTS

Eighteen of the 25 loci did not vary in our survey: Est-4, Gp-1, Gp-3, 6PGD, αGPdh, Hk, Idh, Got, ME, Mdh, Ldh, Adh, Pep-2, G6PD, Pgi-1, Pgi-2, PGM-1, and TO. Seven loci were found to be polymorphic: Est-1, Est-3, Pep-1, Pep-3, CK, PGM-3 and Sdh (Table 1).

None of the loci examined were fixed at alternate alleles. Allele frequencies were heterogeneous among taxa for all polymorphic loci that we examined. There were significant differences from random expectation in the frequencies for all 7 loci (G test, Table 1). For example for CK, the frequency of the a allele was .45 for Orange Cheek, .38 for Orange Lappet and .00 for Big Blue. For Pep-1 the frequency of the a allele was .03 for both Orange Cheek and Big Blue, but .25 for Orange Lappet (Table 1). In addition, no consistent parallel patterns in gene frequencies among different loci for specific pairs of taxa were evident (Table 1).

DISCUSSION

The results of this study indicate that there are no fixed alternate 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 "sibling" species hypothesis (Marsh et al., 1981) or the polymorphic hypothesis (Fryer, 1959). Because electrophoretic divergences are time dependent (Corru_ccini et al. 1980), sibling species that have recently diverged may show little differentiation in electromorphs (Kornfield, 1978).

We conclude, on the basis of both genetic and field evidence, that random mating among the three color morphs of P. tridentiger, in Monkey Bay does not occur. If these had been randomly mating morphs of one species, we would have expected to see homogeneity in gene frequencies among them (Sage & Selander, 1975). The heterogeneity observed in frequencies at all seven polymorphic loci examined is consistent with either of two hypotheses--that these forms represent: 1) isolated and differentiated gene pools or 2) "incipient" species with minimal gene flow between morphs. Whether or not these taxa are: 1) newly evolved totally differentiated "sibling" species, or 2) morphs that are in the process of becoming isolated we feel cannot yet be demonstrated unequivocally. Nevertheless, the genetic information is consistent with the field observations of assortative mating and at least partial differentiation among color morphs. Marsh et al.'s (1981) study of P. tridentiger adds further evidence that coloration is important in species recognition or in mate selection within a species (Eccles & Lewis, 1979; McKaye &

Barlow 1976, Noble & Curtis, 1939; Fryer, 1959, 1977; Greenwood, 1974).

The genetic and field data lend support to the hypothesis that sympatric splitting of morphs could be important in the explosive radiation in the Cichlidae (Kosswig, 1947, 1963; Lowe-McConnell, 1959; Trewavas et al., 1972; Barlow and Munsey, 1976; McKaye, 1978, 1980; Holzberg, 1978; Schroder, 1980). The heterogeneity in allelic frequencies demonstrated here among sympatric taxa parallels other studies in suggesting intial genetic isolation and differentiation occurs without geographic isolation. For example, another polychromatic Malawi cichlid, Pseudotropheus zebra, also has sympatric color morphs that mate assortatively and that form two isolated breeding groups (Holzberg, 1978). Electrophoretic data on the 4 sympatric color morphs of P. zebra show significant differences in allelic frequencies at 4 polymorphic loci (McKaye et al., ms) which are consistent with the mating groups determined by Holzberg (1978). Holzberg (1978) and Schroder (1980) both speculate that the differentiated forms of P. zebra could have arisen sympatrically without geographic isolation.

Maynard Smith's (1966) model of sympatric speciation provides relevant testable predictions for these cases and, possibly for cichlid species flocks in general. The model does not require past geographical isolation of populations. The first stage of Maynard Smith's model is the development of a stable polymorphism. Then, with some degree of differential habitat selection by morphs, two reproductively isolated populations can evolve. Testing

speciation theories is difficult but, if the Maynard Smith model is correct we would expect to see gradations in the degree of differentiation among morphs within a radiating family that lead eventually to total isolation of some forms.

Comparative evidence from the family Cichlidae is consistent with the developmental stages of the sympatric speciation model: 1) polymorphisms without assortative mating (Sage & Selander, 1975); 2) incomplete assortative mating and habitat separation by sympatric morphs (McKaye & Barlow, 1976; McKaye, 1980), and 3) complete assortative mating such that the morphs have achieved specific status (Marsh et al., 1981; Marsh personal communication). Furthermore, sympatric speciation, via an intrinsic behavioral mechanism, should allow more rapid species splitting than allopatric differentiation, via geographic separation and reassociation, does. Sympatric differentiation, thus, may be the most consistent and parsimonious hypothesis to explain some of the cichlid radiation in the Great Lakes of Africa. These and further data from the African cichlids should lead, in addition, to generalizations on alternative modes of speciation fulfilling P. H. Greenwood's (1974) prediction: "In so many respects, the cichlid species flocks are an evolutionary microcosm repeating on a small and appreciable scale the patterns and mechanisms of vertebrate evolution."

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Table 1. Electromorph frequencies for polymorphic loci of Petrotilapia taxa at Monkey Bay, Lake Malawi, Africa. * $p < .05$, ** $p < .01$ (G test)

Taxa	N (alleles)	Allele a	b	c	d	e	f	Heterogeneity G (d.f.)
				Pep-1				
Orange Cheek	38	0.03	0.97					
Orange Lappet	40	0.25	0.75					13.1** (2)
Big Blue	36	0.03	0.97					
				Pep-3				
Orange Cheek	38		1.00					
Orange Lappet	40	0.10	0.90					14.4** (2)
Big Blue	36	0.25	0.75					
				CK				
Orange Cheek	38	0.45	0.55					
Orange Lappet	40	0.38	0.62					31.5** (2)
Big Blue	38		1.00					
				PGM-3				
Orange Cheek	28	0.43	0.54	0.04				
Orange Lappet	36	0.06	0.89	0.06				13.0* (4)
Big Blue	28	0.25	0.75					
				Sdh				
Orange Cheek	38	1.00						
Orange Lappet	40	0.85	0.15					7.3* (2)
Big Blue	38	0.95	0.05					
					Pst-1			
Orange Cheek	38				0.53	0.47		
Orange Lappet	38			0.11	0.53	0.37		13.2* (4)
Big Blue	36				0.75	0.25		

Table 1. (Continued)

Taxa	N (alleles)	Allele a	b	c	d	e	f	Heterogeneity G (d.f.)
				Fst-3				
Orange Cheek	38				0.03	0.66	0.32	
Orange Lappet	38					0.34	0.66	35.0** (4)
Big Blue	36				0.08	0.86	0.06	

Evolution in press

THE CICHLID FISH OF CUATRO CIÉNEGAS, MEXICO: DIRECT EVIDENCE OF CONSPECIFICITY AMONG DISTINCT TROPHIC MORPHS

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Running Head: POLYMORPHISM IN CICHLID FISHES

The idea that different individuals within a biological species may have radically different morphologies and distinct feeding modes has commanded attention because of its profound implications for evolutionary biology. If individuals from a panmitic population are recognized as species by standard taxonomic criteria, organismal diversity in nature may be more apparent than real. This concern is particularly germane to the great diversity seen within assemblages of tropical freshwater fishes as cyprinids and cichlids where endemic specializations are extreme (Myers, 1960; Greenwood, 1974; Reid, 1980). Several examples of substantial dichotomous morphological divergence have been reported among sympatric collections of closely related fishes (Roberts, 1974; Sage and Selander, 1975; Vrijenhoek, 1978; Turner and Grosse, 1980). The cichlids of Cuatro Ciénegas, Mexico are particularly interesting and their study by Sage and Selander set a precedent which has had a significant impact on subsequent systematic studies (Kirkpatrick and Selander, 1979; Graves and Rosenblatt, 1980).

By any phenetic criteria, the extreme distinctiveness of sympatric cichlids in Cuatro Cienegas suggests the presence of two discrete species. A "small tooth" form with papilliform pharyngeal dentition feeds on plant material and possesses a relatively narrow head, slender pharyngeal jaw, and long intestine. A "large tooth" form with molariform pharyngeal teeth feeds on snails and has a wider head, relatively stouter jaw, and short intestine. The morphological and trophic differentiation between these two forms exceeds that observed among many closely related biological species and precisely mimics the differences which define numerous cichlids in the African Great Lakes (Fryer and Iles, 1972).

Further, it is this type of variation that might be associated with incipient macroevolution (Greenwood, 1979). The magnitude of differences between these forms has thus historically caused them to be treated as two distinct species (Taylor and Minckley, 1966; Minckley, 1969; LaBounty, 1974; Kornfield and Koehn, 1975). Alternatively, on the basis of the segregation of both types within wild caught broods, complete electrophoretic similarity, and concordant patterns of allozymic variation between forms, Sage and Selander (1975) suggested that both forms belong to a single polymorphic species. However, this evidence did not provide a definitive test of the two species hypothesis (Hutchinson, 1978).

We present in this article the results of complementary studies of genetics and reproductive biology initiated to resolve the biological status of these fishes. Our allozyme data and observations of mating in natural populations verify the insight of Sage and Selander and attest to their accurate electrophoretic evaluation of conspecificity. This demonstration of substantial morphological variation within a single species has significant implications for ecology and conventional taxonomic characterization.

MATERIAL AND METHODS

Collections of specimens and observations were made in Laguna El Mojarral, Cuatro Ciénegas, Mexico in June - July 1979, 1980 and 1981. Broods of freeswimming offspring and their female guards were collected simultaneously during daylight hours with fine mesh handnets and spear guns. Adult fish for general electrophoretic studies were collected with coarse mesh handnets two hours after sunset when inactive fish rest on the substrate of the laguna.

For electrophoresis, eyes of adult fish were removed and stored frozen in 0.01 M Tris-HCl pH 7.0 (containing 5 mM dithiothreitol and 0.5% polyvinylpyrollidone-360); cichlid fry were frozen whole in buffer. Material was stored for up to three weeks in a conventional freezer (-6°C) while in Mexico, then transferred to an ultracold freezer (-90°C) upon return to the United States. Starch gel electrophoresis was performed as previously described (Kornfield and Koehn, 1975; Sage and Selander, 1975).

Since the morphological identity of reproducing fish could not be established by underwater observation while they were free swimming, it was necessary to capture individuals to determine trophic morphology. Unfortunately, spawning male cichlids were particularly sensitive to the presence of observers in close proximity to breeding areas and fled when approached. To collect actively mating pairs, we designed a remotely operated trap which, when activated, securely captured both male and female fish (Smith et al., 1981). Traps were set out in ecologically uniform habitats where substantial breeding activity had been previously observed. Additional pairs of breeding fish were collected with the use of spear

guns. The pharyngeal dentition of captured fish was determined in the field with an otoscope.

RESULTS AND DISCUSSION

Information bearing on the extent of genetic control over trophic morphology is limited. Both discrete morphological types developed in laboratory reared fish captured as fry from maternally guarded broods (Sage and Selander, 1975). However, it is questionable whether all fry within single groups are sibs; both New and Old World cichlids may protect unrelated offspring (Ribbink et al., 1980; McKaye, 1979). To evaluate this possibility, isolated groups of free swimming fry and their guarding mothers were examined for genetic compatibility at five polymorphic allozyme loci. Of seventeen broods examined, three contained individuals whose electrophoretic phenotypes indicated that they were either unrelated to the females who guarded them or that they had different fathers than other individuals in their broods (Table 1). This assessment of the magnitude of foster parentage is clearly conservative since some unrelated juveniles may be electrophoretically equivalent (see Hanken and Sherman, 1981). Given the allele frequencies characteristic of cichlids at El Mojarral, over one third of all fish at that locality are indistinguishable from the normal offspring of broods GY50 and GY51 for the loci examined. While it is most probable that foreign fry become incorporated into broods by passive mixing (Lewis, 1980), it is also possible that some individuals with paternally incompatible phenotypes were produced by multiple fertilization (Gross and Charnov, 1980; McKaye, 1980). Regardless, it is clear that

inheritance studies based on field-collected offspring can not effectively resolve the systematic problem of the Cuatro Ciénegas cichlids.

Electrophoretic Characterizations

In the absence of breeding information, accurate recognition of separate gene pools can be achieved when sympatric groups exhibit evidence of independent genetic evolution. In the cichlids of Cuatro Ciénegas, estimates of genetic distance are similar to those typical of conspecific populations and do not differentiate the two forms (Kornfield and Koehn, 1975; Sage and Selander, 1975). However, since electrophoretic divergence is probably a time dependent process (Corruccini et al., 1980), very recently evolved species may be expected to be almost indistinguishable. In fish, several studies have demonstrated extreme genetic similarities between valid biological species (Avise et al., 1975; Johnson, 1975); African cichlids in particular exhibit these extremes (Kornfield, 1978; Kornfield et al., 1979).

A more convincing assessment of reproductive isolation involves geographic or temporal comparisons of gene frequencies. Because gene pools of reproductively isolated species respond independently to populational and environmental processes, dissimilar gene frequencies are to be expected (Futuyma and Mayer, 1980). However, frequency differentiation need not be indicative of genetic isolation. For example, significant intraspecific heterogeneity has been reported among age classes in fish (Williams et al., 1973; Koehn and Williams, 1978) and lizards (Tinkle and Selander, 1975). Nevertheless, homogeneity of gene frequencies should generally be characteristic of single species samples regardless of the manner in which individuals are grouped for

comparison. Exploiting this approach, Sage and Selander (1975) demonstrated concordant geographic variation of gene frequencies at several allozyme loci for the two Cuatro Ciénegas cichlids. These observations thus implied the existence of a single gene pool.

We reexamined the frequency of four of these loci previously studied by Sage and Selander in fish from Laguna El Mojarral. At *Eesterase*, *Lactate dehydrogenase-3*, *Phosphoglucoisomerase-1*, and *Phosphoglucoisomerase-2*, genotypic proportions were homogenous among the small tooth and large tooth forms. All individual genotypic ratios fit Hardy-Weinberg-Castle expectations. However, allele frequencies were not temporally stable. Between 1974 and 1980, a significant change in frequency was observed at *PGI-2* (Table 2).

Patterns of coordinated geographic and temporal frequency variation argue against the two species hypothesis. However, it is possible to remain skeptical of this conclusion because of the lack of direct evidence for reproductive isolation. In particular, species may have concordant patterns of allozyme variation in response to common selection pressures (Borowsky, 1977). At single loci, identical patterns of geographic variation in gene frequency have been observed among sympatric populations of reproductively isolated fishes (Johnson, 1974) and molluscs (Koehn and Mitton, 1972; Koehn et al., 1980). It is improbable, of course, that such parallel selective responses occur simultaneously at numerous loci. But if reproductively isolated taxa are of very recent origin, differences in allozyme frequencies may be very limited. Thus, it is essential to examine reproduction under natural conditions to directly test the hypothesis of two biological species.

Reproductive Observations

Isolated gene pools evolve independently only if significant gene flow does not occur between them (Crow and Kimura, 1970; Jackson and Pounds, 1979). A limited amount of interspecific gene exchange can be tolerated and still maintain reproductive isolation if selection is relatively strong. In general, though, the number of interspecific matings in sympatric populations should approach zero.

In Cuatro Ciénegas, actively reproducing male-female cichlid pairs were identified by observing standard behaviors of spawning and nest guarding (Baerends and Baerends-Van Roon, 1950). A sample of 33 reproductively active pairs was collected in Laguna El Mojarral during 1979, 1980 and 1981. If the two forms were completely isolated reproductively, we would have expected to see no matings between forms. Examination of the pharyngeal jaws revealed that 19 mating pairs (57%) involved fish with unlike trophic morphologies (Table 3). Further, given the relative proportion of the small tooth cichlid in this population, the observed number of homotypic matings was much less than that expected under reproductive isolation. Mixed morphological matings were also observed at three additional localities: Pozo de la Becerra, Laguna Churince, and Rio Mesquites. Clearly these observations force rejection of the two species hypothesis.

Although the data presented did not deviate from random mating expectations ($G = 0.244$, $df = 1$, $p > .5$), the sample size does not preclude limited assortative mating by morphotype. In fact, if almost 20% of all matings were completely assortative, we would have been unable to demonstrate this given the small sample size. However, assortative mating is unlikely. First, our field observations of

natural reproduction did not reveal any obvious habitat segregation. Matings in Laguna El Mojarral occurred on an ecologically monotonous substrate in close proximity to one another. Second, males who sequentially fertilized the eggs of separate females and simultaneously guarded two nests exhibited no mating fidelity to fish of their own trophic type. Two of five males who maintained distinct nests were mated to both small tooth and large tooth females concurrently.

Implications

The confirmed existence of a trophically dichotomous biological species impacts on two distinct areas of evolutionary biology. First, the variation observed between cichlid morphs, and that recently noted in goodeids by Turner and Grosse (1980), forces a critical appraisal of the concept of adaptive radiation; trophic polymorphism may represent a viable alternative evolutionary strategy. The factors controlling the origin of such a system remain unclear. Anatomical flexibility (Liem, 1980) and/or competition and resource availability may constrain the formation of trophic polymorphisms. Need we reevaluate the niche concept to accomodate such species (Hutchinson, 1978)? Surely their ecological behavior differs significantly from conventional taxa. For example, simple communities composed of trophically polymorphic species may be more responsive to environmental and biotic perturbations than standard communities.

Second, the range of dichotomous morphological variation between cichlid morphs has profound systematic implications. Numerous species exhibit morphological modifications of the pharyngeal dentition and apophysis mirroring the type observed in Cuatro Ciénegas (Greenwood,

1974, 1979; Hoogerhond and Barel, 1978). If species definitions are primarily based on such phenetic differences, morphology by itself may be generally inappropriate to delimit biological taxa or index actual species diversity. It is of considerable importance that cichlid systematists have begun to emphasize breeding coloration (Barel et al., 1977; Greenwood and Barel, 1978). Cichlid species generally appear to be distinguished by unique coloration (Greenwood, 1974) and assortative mating by color has recently been described for a number of taxa formerly thought to be color morphs of single species (Holzberg, 1978; Schroder, 1980; Marsh et al., 1981). However, color differences, like morphological differences, may not be sufficient to recognize reproductively isolated taxa. In Lake Malawi, trophically equivalent cichlids which differ significantly in breeding coloration and body shape were statistically indistinguishable in allele frequency at three polymorphic isozyme loci (Kornfield, 1974). Clearly resolution of such systematic problems will require direct observations of reproductive isolation or more sophisticated probes for genetic continuity (e.g. Avise et al., 1979). Regardless, differentiation without speciation has received only limited attention by taxonomists. Since this situation exists in fish, it is not unreasonable to suggest that it may occur in additional vertebrate groups.

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SUMMARY

Two discrete cichlid fishes endemic to Cuatro Ciénegas, Mexico differ significantly in several morphological characters associated with feeding specializations. Conventional taxonomic treatment would suggest the presence of two distinct species, but Sage and Selander (1975) forcefully argued for the presence of a single polymorphic biological species. Biochemical comparisons and observations of reproductive in situ were conducted in 1979, 1980 and 1981 to reevaluate the two species hypothesis.

Electrophoresis of juvenile cichlids and their brooding mothers revealed individuals with parentally incompatible phenotypes. Thus, the inheritance of morphology inferred from segregation of individuals within wild caught broods is questionable. Electrophoretic examination of four polymorphic loci revealed gene and genotypic homogeneity between the two types of fishes. A significant change in allele frequency at *PGI-2* which occurred between 1974 and 1980 was observed in both forms. This temporal concordance in gene frequency between forms inferentially argued against the two species hypothesis.

To directly examine reproductive isolation, cichlid pairs were collected in the process of mating. Few matings between the two forms were expected under the two species hypothesis. Of 33 reproductive pairs examined, 57% involved matings between forms. These observations unequivocally suggest the existence of a single Mendelian population.

Confirmation of a trophically polymorphic biological species has significant ecological and systematic implications. Trophic polymorphism may represent an alternative strategy to adaptive radiation and may inflate estimates of species diversity based solely on phenetically defined taxa.

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Table 1. Foreign phenotypes of fry in guarded cichlid broods.

Family	Number of fry	Phenotype	*LDH-1*	*LDH-2*	*LDH-3*	*PGI-1*	*PGI-2*
		Mother	-	-	FF	FF	SS
GY50	145	Offspring	-	-	FF	FF	SS
		Foreign Fry No. 1	-	-	FF	FF	FF
		Foreign Fry No. 2	-	-	FF	FF	SF
		Mother	-	-	FF	FF	SS
GY51	54	Offspring	-	-	FF	FF	SS
		Foreign Fry No. 1	-	-	FF	FF	SF
		Mother	-	-	-	-	-
GY01	74	Offspring	SF/FF	SS	FF	FF	SF
		Foreign Fry No. 1	SS	SF	SF	SF	SF
		Foreign Fry No. 2	SF	SS	SS	FF	SF

Phenotypes of fry in bold type are parentally incompatible. Relative allele mobilities in Sage and Selander (1975).

Table 2. Frequency estimates of *PGI-2* (*1.00*) in samples of cichlids from Laguna El Mojarral.

	Small tooth		Large tooth		Total	
	N	p ± s.e.	N	p ± s.e.	N	p ± s.e.
1974	20	0.70±.072	15	0.57±.090	52	0.63±.047
1980	30	0.417±.064	28	0.428±.066	58	0.422±.046

Population estimates of allele frequency differ significantly between years ($t = 2.20$, $p < .05$). Data from 1974 from Sage and Selander (1975).

Table 3. Observed association of trophic morphology in breeding pairs of Mexican cichlids.

		Male	
		Papilliform	Molariform
Female	Papilliform	13 (11.85)	10 (11.15)
	Molariform	5 (5.15)	6 (4.85)

Expected number of pairs under the random mating hypothesis in parentheses. Note the difference in the relative frequencies of the tooth types between sexes.

In press

INHERITANCE OF ALLOZYMES IN ATLANTIC HERRING (*CLUPEA HARENGUS HARENGUS*)

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ABSTRACT

Progeny from single pair crosses of Atlantic herring were examined to determine the heritability of genetic variation at seven polymorphic allozyme loci. Mendelian inheritance of codominant autosomal alleles was established for *IDH-2*, *LDH-1*, *LDH-2*, *ME-2*, *PGM-1*, *PGM-2*, and *PGI-2*. This demonstration of Mendelian inheritance is essential for accurate interpretaion of allozymic variation among natural populations of this pelagic species.

INTRODUCTION

Formal genetic studies of allozyme variation in pelagic fishes are infrequent because of technical problems associated with live capture and artificial culture of reproductively mature fish and progeny. However, such studies are central to correct interpretation of allozyme variation in natural populations. On the basis of electrophoretic patterns and statistical fits to Hardy-Weinberg-Castle expectations, variation is typically assumed to be inherited in a simple Mendelian manner. But this assumption can be wrong (Spiess, 1977; Fairbairn and Roff, 1980); a variety of nongenetic factors may alter electrophoretic patterns or phenotypic frequencies of populations.

Before completing electrophoretic studies designed to discriminate stock composition among Atlantic herring (*Clupea harengus harengus*), we performed single pair artificial matings and reared progeny in laboratory culture to determine the inheritance of allozymes. These findings significantly enhance our ability to interpret accurately allozyme differences in this species.

MATERIAL AND METHODS

Reproductively mature adult herring were selected from freshly purse-seined fish on 15 October 1980, east of Plum Island, Massachusetts (42°24'N; 71°30'W). *In vitro* single pair fertilizations were performed following methods outlined by Blaxter (1968). Larvae from 41 individual crosses were maintained at 15°C for 14 days in constantly aerated synthetic seawater (salinity 27 ppt).

Liver and muscle tissues dissected from parents were buffered with an equal volume of 0.01M Tris-HCl pH 7.0 (containing 5mM dithiothreitol and 0.5% polyvinylpyrollidone-360) and frozen in liquid nitrogen. Larvae and adult tissues were subject to horizontal starch gel electrophoresis using methods described by May *et al.* (1979). Enzymes assayed, adult tissue distributions, and electrophoretic buffer systems are presented in Table I. For each locus, the most frequent anodal allele in natural populations was assigned a relative mobility of 100. Observed and expected genotypes were tested for homogeneity by G-test with Yates' correction (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

With the exception of *PGM-2*, polymorphism has been previously observed at all other loci in populations of Atlantic herring. Observed genotypes and Mendelian expectations for crosses involving seven allozyme loci are presented in Tables II and III. Many crosses involved repetitive examination of matings between parents with identical genotypes. The results shown here encompass all genotypes involved and present those crosses which exhibited maximum deviation from Mendelian expectations. Progeny from at least one additional mating were examined for each type of cross shown. Because fertilizations were initiated without knowledge of parental genotypes, only homozygote x homozygote crosses could be assayed for *AAT*, *EST*, *ME-1*, *PGI-1*, *SOD*. For these loci, electrophoresis of progeny from all crosses revealed only expected homozygous genotypes. The data of Tables II and III and the presence of multibanded heterozygote genotypes in both sexes for all polymorphic enzyme loci indicate that the observed variants are autosomal

codominants and are inherited in a simple Mendelian manner.

Electrophoretic patterns of some dimeric and tetrameric enzymes exhibited a reduced number of isozymes between loci. The absence of interlocus heteromeric isozymes for malic enzyme (*ME-1* and *ME-2*) and isocitrate dehydrogenase (*IDH-1* and *IDH-2*) may indicate substantial evolutionary divergence in structure or composition of component loci. Only a single interlocus heterotetrameric isozyme was observed between *LDH-1* and *LDH-2* homozygotes. However, heterozygotes at each locus exhibited typical 5 banded phenotypes. The absence of two additional isozymes expected to form between these loci has been observed in other fishes and may result from restricted subunit assembly (Whitt and Horowitz, 1970).

A variety of independent factors may contribute to nongenetic alteration of electrophoretic mobility. The most common type of change is post-translational and involves covalent modifications of peptide bonds, terminal groups, and side chains (Harris and Hopkinson, 1976; Uy and Wold, 1980). Many such examples of posttranslational alteration have been established. When this type of modification is controlled by a polymorphic regulatory locus, alleles at affected enzyme loci may not be expressed in a codominant manner (Cochrane and Richmond, 1979). Changes in electrophoretic patterns can also be generated for many loci by storage of tissues for prolonged periods. Oxidation of sulfhydryl groups can occur spontaneously to affect the formation of disulfide bridges (Uy and Wold, 1977). Additional nongenetic modification of electrophoretic phenotypes has been associated with physiological variation (Oki *et al.*, 1966) and parasitism (Vrijenhoek, 1975). The results of our inheritance tests indicate that none of these factors influenced phenotypes in our material.

Significant errors in the interpretation of allozyme variation

observed in natural populations can be produced when simple genetic models are uncritically accepted. Entirely spurious hypotheses of population structure may be generated as a result of an inappropriate model (Fairbairn and Roff, 1980). Since conclusions from electrophoretic studies can significantly affect management decisions for commerical fisheries, formal genetic analyses are essential. The Mendelian inheritance of allozyme variation in Atlantic herring presented here will enable us to address more accurately problems of population structure in this important commercial species.

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Table I.
Enzyme systems studied in *Clupea harengus*

Enzyme (E.C. number)	Abbreviation	No. of loci	Subunit structure	Adult tissue	Buffer system[1],[2]
Aspartate aminotransferase (2.6.1.1)	AAT	1	dimeric	muscle	I
Esterase (3.1.1.1)	EST	1	monomeric	liver	I
Isocitrate dehydrogenase (1.1.1.41)	IDH	2	dimeric	muscle	II[3]
Lactate dehydrogenase (1.1.1.27)	LDH	2	tetrameric	muscle	I
Malic enzyme (1.1.1.40)	ME	2	tetrameric	muscle	II
Phosphoglucoisomerase (5.3.1.9)	PGI	1	dimeric	muscle	I
Phosphoglucomutase (2.7.5.1)	PGM	2	monomeric	liver	I[4]
Superoxide dismutase (1.15.1.1)	SOD	1	dimeric	liver	I[5]

[1] Buffer system I (Ridgway et al., 1970); II (Clayton and Tretiak, 1972)

[2] ATT stained according to Johnson et al. (1972); all other enzymes used histochemical stains from Shaw and Prasad (1970) with an agar overlay (Brewer, 1970).

[3] Add $MgCl_2$ to staining mixture, omit $MnCl_2$

[4] Add 1 mg glucose-1, 6-diphosphate to staining mixture

[5] Stains incidentally with PGM

Table II.
Inheritance of diallelic allozyme loci in *Clupea harengus*

Locus	Relative allele mobility	Cross number	Parental genotype	Number of progeny	Observed genotypes AA	AB	BB	Expected genotypes AA	AB	BB	G	P
IDH-2	A = *100*	19	AA x AA	55	55	0	0	55	0	0	-	
	B = *113*	35	AA x AB	46	25	21	0	23.0	23.0	0	0.098	0.754
LDH-2	A = *100*	21	AA x AA	54	54	0	0	54	0	0	-	
	B = *172*	28	AA x AB	57	26	31	0	28.5	28.5	0	0.140	0.708
LDH-1	A = *0*	38	AA x AA	52	52	0	0	52	0	0	-	
	B = *100*	31	AA x AB	57	31	26	0	28.5	28.5	0	0.140	0.708
ME-2	A = *100*	21	AA x AA	54	54	0	0	54	0	0	-	
	B = *35*	17	AA x AB	55	31	24	0	27.5	27.5	0	0.328	0.567
PGM-1	A = *100*	12	AA x AA	53	53	0	0	53	0	0	-	
	B = *125*	33	AA x AB	49	22	27	0	24.5	24.5	0	0.163	0.686

Table III.
Inheritance of triallelic allozyme loci in *Clupea harengus*

Locus	Relative allele mobility	Cross number	Parental genotype	Number of progeny	Genotypes[1] AA	AB	BB	AC	BC	CC	G	df	p
PGI-2	*A = 100*	31	AA x AA	57	57	-	-	-	-	-	-	-	
	B = 150				57	-	-	-	-	-	-	-	
	C = -75												
		6	AA x AC	54	25	29	-	-	-	-			
					27	27	-	-	-	-	.083	1	.773
		23	AB x AC	48	11	14	-	13	10	-			
					12	12	-	12	12	-	.418	3	.936
PGM-2	*A = 112*	31	BC x BC	57	-	-	13	-	27	17			
	B = 100				-	-	14.25	-	28.5	14.25	.346	2	.841
	C = 92												
		33	BB x BC	55	-	-	25	-	30	-			
					-	-	27.5	-	27.5	-	.145	1	.703
		24	AC x BC	52	-	11	-	15	12	14			
					-	13	-	13	13	13	.386	3	.943
		23	AB x CC	49	-	-	-	23	26	-			
					-	-	-	24.5	24.5	-	.040	1	.841

[1] Observed genotypes listed above expected for each cross

In review

Stock definition in Atlantic herring: genetic evidence for discrete fall and spring spawning populations

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Ripe Atlantic herring were sampled from seven discrete spawning grounds in the Gulf of Maine and Gulf of St. Lawrence over a period of three years. Genetic polymorphisms were observed at 13 enzyme loci by starch gel electrophoresis. Five highly polymorphic loci were used to assess population structure of herring stocks by contingency analysis of log-likelihood differences in gene frequencies. Significant heterogeneity was observed within both spring spawning and fall spawning populations for particular years, but was not significant for either season after adjusting for temporal instability. By contrast, overall heterogeneity between spring and fall spawning populations was highly significant. These results indicate genetic isolation of spring spawning populations in the Gulf of St. Lawrence from fall spawning aggregates in the Gulf of St. Lawrence and Gulf of Maine. The low levels of genetic heterogeneity and absence of temporal stability of gene frequencies in fall spawning stocks are not consistent with the existence of more than a single genetic population of fall spawning herring in the northwest Atlantic.

Key words: *Clupea harengus harengus*, population genetics, biochemical genetic variation, stock differentiation.

Atlantic herring (*Clupea harengus harengus* L.) are a significant resource along the eastern coast of Canada and the United States. The species has been continuously exploited since pre-colonial times when prodigious populations were commonly observed in coastal communities (Bigelow and Schroeder 1953). More recent biomass fluctuations, possibly resulting from intense multinational fishing efforts, have created a need for accurate information on the population biology of this species. More specifically, information on the extent of reproductive isolation among spawning groups and the extent of population mixing during non-reproductive periods is essential for species management.

Major features of the biology and life cycle of Atlantic herring have been established (Blaxter and Holliday 1963), but detailed life history characteristics are poorly understood. In the western North Atlantic, reproduction is restricted to two intensive bouts, one in the fall (September-November) and one in the spring (April-May). At these times, large numbers of mature adults spawn at relatively discrete geographic locations (Boyar et al. 1973). Along the northeast coast of North America, spring spawning aggregations are currently restricted to localities in and around the Gulf of St. Lawrence, whereas fall spawning occurs in numerous areas, particularly the Gulf of Maine and the Gulf of St. Lawrence. Individual spawning aggregations have historically been considered by fisheries managers to be distinct entities, i.e. stocks. Fish tagged in the Gulf of Maine, Georges Bank and Nova Scotia mix during non-spawning migratory periods (Spiers 1977; Stobo 1976). However, the actual extent and duration of population mixing during various stages of the life cycle of herring is unknown and has been the point of repeated speculation (Anon. 1978; Vernberg 1977). While adults annually concentrate at specific locations to spawn, the fidelity of particular stocks to a specific spawning

area is unknown. If, as has been widely assumed, fidelity of returning adult fish to spawning sites is essentially absolute, individual stocks would represent discrete reproductively isolated populations.

Central to this problem is the historical fisheries biology concept of a fish "stock" as an exploitable unit, which may or may not include members of different genetic populations (Larkin 1972). Yet, efficient exploitation of multiple stocks by a common fishery is based upon the assumption that stocks are reproductively isolated (Paulik et al. 1967), i.e. a stock is a genetic population. The problem of population definition and gene flow must be therefore approached from a strictly genetic veiwpoint. The only period in the life history of the herring where it is possible to examine reproductive isolation is during spawning. Because of mixing at other life history stages, geographic comparisons during non-spawning periods are of no value in delimiting populations (Williams et al. 1973). To define populations, it is critical that only spawning fishes be examined.

Fisheries scientists have attempted to use a variety of techniques to discriminate populations including internal and external morphology, biological tags, and protein variants. Meristic characters have been employed with varying success. Otoliths have been used to convincingly demonstrate significant differences between spring and fall spawning populations in the Gulf of St. Lawrence (Côté et al. 1980). Despite successful characterization of stocks by these methods, variation in morphological characters may have a dominant environmental component. High degrees of morphometric similarity between spawning aggregations may result if environmental conditions influencing these characters are similar; conclusive evidence of biological isolation among populations requires demonstration of genetic differences (Messieh and Tibbo 1971).

For over twenty years, biologists have studied biochemical variation in Atlantic herring in attempts to define population structure. Because it provides genetic identification without confounding environmental influences, biochemical variation as assayed by electrophoresis is a powerful tool for population discrimination and identification. Previous studies have identified a portion of the biochemical genetic variation in Atlantic herring (Mairs and Sindermann 1960; Sindermann and Honey 1963; Odense et al. 1966; Wilkins and Iles 1966; Lush 1969; Naevdal 1969a,b; Simonarson and Watts 1969; Ridgway et al. 1970; Wolf et al. 1970; Engel et al. 1971; DeLigny 1972; Lewis and Ridgway 1972; Odense et al. 1973; Odense 1980). However, these studies are of limited value in assessing population structure because they did not meet two essential criteria: 1) the genetic basis (i.e. heritability) of observed variation must be established by breeding experiments (Fairbairn and Roff 1980), and 2) only sexually mature fish in spawning condition must be sampled from discrete spawning grounds.

This paper presents the results of an intensive study to characterize the genetic variation within and among geographically distinct samples of Atlantic herring. A large number of proteins were resolved from which thirteen polymorphic loci were extensively examined in reproducing adults at specific spring and fall spawning localities over a three year period. We feel that this study represents a rigorous examination of northwest Atlantic herring population structure because: 1) the Mendelian heritability of the polymorphic loci employed was established through breeding experiments (Kornfield et al. 1981); 2) only sexually competent fish from known spawning aggregates were sampled; and 3) the temporal stability of gene frequencies at all spawning/sampling sites was tested over two (in some cases, three) consecutive years.

MATERIAL AND METHODS

Samples of Atlantic herring were collected in conjunction with commercial fisheries at seven spawning locations in the Gulf of Maine and Gulf of St. Lawrence (Fig. 1; Table 1). Two locations were sampled only in 1979, three in 1979 and 1980, and two in 1978, 1979, and 1980. Collections were made during the peak of reproduction at discrete spawning areas; all fish were ripe. Samples of liver and muscle tissue were removed from individuals directly after capture and frozen in liquid nitrogen. Tissues were later transferred to an ultracold freezer (-80°C) and stored for up to six months prior to electrophoresis. Samples of Pacific herring (*C.h. pallasi*) in non-spawning condition were obtained from Puget Sound.

Tissues were homogenized in an equal volume of cold 10 mM Tris-HCl pH 7.0 (containing 5 mM dithiothreitol and 0.5% polyvinylpyrrolidone-360) and centrifuged at 2000 X g for five minutes. Horizontal starch gel electrophoresis of supernatants followed the procedure outlined by May et al. (1979). For most proteins, histochemical staining followed the methods of Shaw and Prasad (1970) and Harris and Hopkinson (1977). Aspartate aminotransferase was visualized by the method of Johnson et al. (1972). Of 38 proteins initially screened, 29 enzymes (Table 2) were examined for biochemical variants.

Designation of isozymes and genotypes follows the uniform nomenclature suggested by Allendorf and Utter (1979). For statistical interpretation of observed genetic variation, we employed contingency analysis of the log-likelihood procedure (Smouse and Ward 1978) adjusting the significance of probability tests for individual loci (Grant and Utter 1980; Grant 1981).

Estimates of genetic similarities among sampled populations were calculated with the formulae of Nei (1972).

RESULTS

We electrophoretically resolved 42 gene loci encoding 29 proteins. All of these loci were initially examined in a sample of 100 individuals from a single population (Jefferies Ledge) to characterize polymorphisms for subsequent population screening. Of the 20 proteins that exhibited monomorphic loci in this sample, eleven were electrophoretically compared with samples of Pacific herring. In this subsequent comparison, 9 of these 11 proteins exhibited divergent alleles at a minimum of one locus per protein. Thus, we are confident that our techniques were capable of detecting a significant proportion of electrophoretic variation that might have been present in our initial screening.

Electrophoretic variants were observed at 13 loci encoding 9 enzymes (Fig. 2). However, variation was rare ay many of these loci. Allelic frequencies for the most informative loci, i.e. those where the frequency of most common allele $\leq .95$, are presented in Tables 3 and 4. Allelic frequencies for all other variable loci are provided in the Appendix. A brief characterization of each polymorphic system follows.

ACON -- *ACON* is encoded by two anodal loci of which the more anodal, *ACON-2*, was polymorphic and possessed five alleles. Two-banded heterozygotes implied monomeric structure of the active isozyme. Care must be taken in staining because the linking enzyme in the stain is very sensitive to heat denaturation; *ACON* itself is quite labile. Variation for *ACON* has not been previously reported in Atlantic herring.

AAT -- A single anodal locus segregating for three alleles was observed. 1:2:1 staining intensities observed in the three-banded heterozygote phenotypes imply that the locus is dimeric. Crosses among *AAT* homozygotes

produced only homozygous offspring (Kornfield et al. 1981). Variation at this locus has been previously reported in Atlantic herring from eastern North America and Western Europe (Odense et al. 1966, 1973).

EST -- Esterase is coded for by several loci, only the most anodal of which was interpretable. This fast locus was polymorphic with six alleles observed. The presence of two-banded heterozygotes implied a functional monomer. Crosses among *EST* homozygotes produced only homozygous progeny (Kornfield et al. 1981). Variation of esterase in Atlantic herring has previously been reported (Naevdal 1969; Simonarson and Watts 1969; Ridgway et al. 1970; Odense et al. 1973).

IDH -- Two anodal polymorphic loci were observed. We have demonstrated Mendelian inheritance of allelic variation for *IDH-2* (Kornfield et al. 1981). Variation at *IDH* in Atlantic herring has been reported by Wolf et al. (1970).

LDH -- Three loci were observed, however only two of these (*LDH-1* and *LDH-2*) were scored in all populations. Mendelian inheritance of alleles has been demonstrated for both of these loci (Kornfield et al. 1981). At *LDH-1*, the common allele is situated on the origin of the gel and is assigned mobility *0*. Variation at *LDH* in the Atlantic subspecies has been previously reported (Odense et al. 1966, 1973).

ME -- Allelic variants were observed at two anodal loci. Mendelian inheritance has been established for *ME-2* (Kornfield et al. 1981). Tetrameric structure is consistent with the observed heterozygote phenotype. A rare variant of *ME-2 (55)* was equivalent in mobility to the common *100* allele at *ME-1* (Figure 2). Variation at these loci has not been previously reported in Atlantic herring.

PGI -- One polymorphic anodal locus (*PGI-2*) and one monomorphic cathodal

locus (*PGI-1*) were studied. Allelic variation at *PGI-2* is inherited in a Mendelian manner (Kornfield et al. 1981). The single *PGI-1* allele was equivalent in mobility to the *-75* allele of *PGI-2*. No heterodimers occur between loci. *PGI-2* was highly polymorphic exhibiting a total of 14 anodal and cathodal alleles throughout the range studied. Many of these alleles were rare variants restricted to single localities. Two null alleles were encountered in herring sampled from Miscou. Compared to the 1:2:1 staining intensities of normal three-banded heterozygotes, null heterozygotes were recognized as phenotypes which exhibited two bands of equal staining intensity representing the normal/normal homodimer and the normal/null heterodimer. Mobilities of null alleles were calculated as twice the distance between the normal homodimer and the normal/null heterodimer; null homodimers are represented by dotted lines in Figure 2. Variation at *PGI-2* in Atlantic herring has been previously described (Lewis and Ridgway 1972; Odense et al. 1973).

PGM -- Two polymorphic anodal loci were studied. Both loci exhibited Mendelian inheritance of allelic variants (Kornfield et al. 1981). Variation in *PGM-2* has been described in western European samples of Atlantic herring (Lush 1969).

SOD -- A single anodal locus polymorphic for 4 alleles was studied. Crosses among *SOD* homozygotes produced only homozygous progeny (Kornfield et al. 1981). Variation for *SOD* has not been previously reported.

Among the twelve samples, the average proportion of loci heterozygous per individual was 0.048 and average proportions of loci polymorphic per sample were 0.109 and 0.213 for the 95% and 99% polymorphism criteria, respectively (Table 1). With only a single exception, <u>all</u> samples were in Hardy-Weinberg-Castle equilibrium for <u>all</u> polymorphic loci. Observed

genotypes for *EST* in the 1980 herring sample from Jefferies Ledge deviated significantly from equilibrium expectations (pooling alleles *102* and *104*, $\chi^2 = 11.53$, df = 3, $p < 0.01$).

Allele frequencies were examined to estimate the magnitude of differentiation among herring populations. Heterogeneity among samples was tested by the log-likelihood procedure in a hierarchical manner for the five loci polymorphic under the 95% criterion (*ACON-2*, *EST*, *LDH-2*, *PGI-2*, and *PGM-2*). Tests associated with individual loci were summed and examined for overall significance. We examined variation at each locus (1) within years for spring and fall samples separately, (2) between spring and fall for specific years, and (3) between spring and fall among years. Results are summarized in Table 5.

Significant heterogeneity within spring spawning populations was only observed in 1980 for *PGM-2* ($p < 0.01$) and for the likelihood SUM over all loci ($p < 0.05$). Heterogeneity among fall spawning samples was observed for one locus in 1978 (*ACON-2*, $p < 0.05$; SUM, $p < 0.05$) and a different locus in 1979 (*PGI-2*, $p < 0.05$; SUM, $p < 0.001$). Heterogeneity between spring and fall spawning samples was observed in both 1979 (*ACON-2*, $p < 0.01$; SUM, $p < 0.001$) and 1980 (*PGM-2*, $p < 0.05$; SUM, $p < 0.001$). Overall heterogeneity between spring and fall spawning populations was highly significant ($p < 0.001$) for the locus SUM and was significant at three of the five polymorphic loci examined (*ACON-2*, $p < 0.01$; *EST*, $p < 0.05$; *PGI-2*, $p < 0.01$).

It is not appropriate to interpret the significant difference between spring and fall samples without correcting for the variation which occurred within seasons. To characterize this difference, we generated an approximate F test as the ratio of (1) likelihood / degrees of freedom for the

sum of all loci for all samples divided by (2) the pooled likelihood estimate / pooled degrees of freedom for the sum of all loci among spring spawning and among fall spawning samples. A highly significant overall difference between spring and fall spawning areas was revealed by this test ($F = 5.54$; $df = 10, 30$; $p < 0.001$). In a similar manner we separately tested the total temporal variation among spring spawning populations and among fall spawning populations correcting for variation within years; both of these F tests were non-significant.

Genetic similarities among samples calculated on the basis of the 13 variable loci all exceeded $I_N = 0.994$. To provide better characterization of intersample identity, we recalculated similarities based only on the highly polymorphic loci (*ACON-2*, *EST*, *PGM-2*, and *PGI-2*). Similarities were again very high ranging from 0.969 to 0.998 (Table 6). In this restricted set of loci, average similarities within spring and fall samples were marginally higher (0.9927 and 0.9911) than similarities between seasons (0.9899). However, some similarities between particular spring-fall pairs were extremely high.

DISCUSSION

Attempts to define populations of Atlantic herring have had a long and controversial history. While several workers have considered spring and fall spawning herring to represent a single population (Jean 1956; Tibbo and Graham 1963), a large number of fishery biologists have supported the idea of separate stocks (Prince 1907; Tibbo 1957; Day 1957; Messieh and Tibbo 1971; Messieh 1975; Cote et al. 1980). Biologists have been less certain about the genetic uniqueness of individual spawning aggregations, but a few authors have suggested that genetic isolation exists between stocks (Ridgway et al. 1970; Lewis and Ridgway 1972; Zenkin 1978). However, because non-spawning fish were examined in many of these studies and characters which could potentially be environmentally influenced were employed, it is difficult to explicity make a test for genetic isolation. Our examination of inherited biochemical variation in spawning herring aggregations circumvents these weaknesses.

It is clear from the likelihood analysis of genetic variation (Table 5) that spring spawning herring are genetically differentiated from fall spawners. They thus possess isolated gene pools which can respond independently to ecological variation or fishing pressure. However, the magnitude of differentiation between these groups is small and implies that they are probably of relatively recent common origin. More importantly, statistically significant differences between seasonal spawners do not provide suitable biochemical markers for accurate discrimination of individual fish. Statistical separation also does not preclude limited gene flow between populations spawning in different seasons. For example, while morphological discriminate functions convincingly separated fall and

spring spawning herring examined by Cote et al. (1980), approximately 5% of spawning individuals were misclassified. Such an error could indicate the magnitude of gene flow between seasonal isolates, though other factors likely contribute to incorrect identification. The absence of consistent heterozygote deficiencies at polymorphic loci within spring spawning samples also suggests extremely little interseasonal gene exchange. Nevertheless, the extent of gene flow must be very small or genetic differences could not persist (Aspinwall 1974).

The existence of additional genetically isolated or semi-isolated populations within spring or fall spawning groups is less clear. Yearly variation in gene frequencies at specific sampling sites tends to mask differences that might exist among separate spawning localities. The magnitude of this annual variation is of considerable importance. Temporal stability of gene frequencies is one of the essential prerequisites for realistic use of electrophoretic variation in defining populations (Allendorf and Utter 1979; Utter et al. 1980). Had we only sampled in 1978 or 1979, we would have incorrectly interpreted the significant difference observed among separate fall spawning localities as indicative of several genetically-isolated populations. However, because of frequency homogeneity among samples collected from the same spawning localities in 1980, it is clear that significant heterogeneity among sample localities is temporally unstable and may represent stochastic sampling error.

On the basis of gene frequency differences at *EST* and *PGI-2* in non-spawning herring, Ridgway et al. (1970) and Lewis and Ridgway (1972) suggested the existence of separate herring subpopulations in the Gulf of Maine. It is of interest that *PGI-2* is one of several polymorphic loci of herring (including *AAT*, *IDH-2*, *LDH-1* and *LDH-2*) which exhibit no

significant frequency differences between western Europe and eastern North America (Wolf et al. 1970; Odense et al. 1973). Given such oceanic homogeneity and the errors associated with comparing non-spawning herring from a single year, this conclusion of genetic isolation seems unjustified. Previously, Anthony and Boyar (1968) noted significant differences in pectoral ray and vertebral number among samples of Atlantic herring from the northwest Atlantic. They concluded that two general complexes of herring exist within the Gulf of Maine. Meristic differences observed by them between the two years sampled were interpreted as indicating a change in the distribution of herring. However, variation in meristic characters may be induced by different thermal regimes during development (Day 1957; Ali and Lindsey 1974; Dentry and Lindsey 1978). We suggest that the absence of temporal consistency in meristic data does not support a multipopulation model.

Our results are not consistent with the existence of more than a single population of fall-spawning herring in the Gulf of Maine. This finding contrasts with that in the more polymorphic Pacific subspecies where significant geographic heterogeneity has been identified (Grant 1981). While it is possible that spawning aggregations of Atlantic herring are reproductively isolated within a particular season, the genetic differences among populations must be extremely small. We suggest that more sophisticated biochemical techniques, e.g. Ramshaw et al. 1979, Smith et al. 1981, will be of little value to detect such differences among populations, if in fact these differences exist.

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Table 1. Collection localities and estimates of genetic variability in samples of Atlantic herring.

Geographic location		Date Collected		Average no. individuals per locus	Proportion of loci Polymorphic per population[a]	Proportion of loci Heterozygous per individual
SPRING SPAWNING LOCALITIES						
Carleton, N.S.	48°10'N;66°00'W	14 May 1979	C79	43.9	0.143	0.044
		6 May 1980	C80	78.5	0.095	0.046
Ile Verte, Que.	48°00'N;69°30'W	13 May 1979	IV79	66.5	0.095	0.048
		5 May 1980	IV80	98.0	0.095	0.044
FALL SPAWNING LOCALITIES						
Jefferies Ledge, MA	42°40'N;70°20'W	2 October 1978	JL78	79.8	0.095	0.050
		1 October 1979	JL79	102.8	0.119	0.053
		5 October 1980	JL80	98.6	0.117	0.050
Lurcher Shoals, N.S.	43°50'N;66°30'W	21 September 1978	LS78	74.0	0.095	0.040
		4 October 1979	LS79	90.2	0.095	0.047
		12 October 1980	LS80	94.5	0.119	0.046
Miscou, Que.	48°10'N;64°20'W	22 September 1979	M79	47.0	0.119	0.059
Prince Edward Islad, P.E.I.	47°10'N;64°00'W	25 September 1979	PEI79	96.2	0.119	0.048
Stonehaven, N.B.	47°50'N;65°20'W	22 September 1979	S79	40.8	0.119	0.049
		4 October 1980	S80	95.5	0.095	0.050

[a] A locus is considered polymorphic if the frequency of the most common allele is ≤ 0.95.

Table 2. Proteins resolved and electrophoretic methods for *Clupea harengus*.

Protein	Number of loci	Tissue	Buffer[a]
Aconitase (*ACON*; E.C.4.2.1.3)	1	Liver	3
Aspartate amino transferase (*AAT*; E.C.2.6.1.1)	1	Muscle	1
Alcohol dehydrogenase (*ADH*; E.C.1.1.1.1)	2	Liver	3
Alphaglycerophosphate dehydrogenase (*AGP*; E.C.1.1.1.8)	3	Muscle	2
Creatine kinase (*CPK*; E.C.4.2.1.11)	2	Muscle	3
Esterase (*EST*; E.C.3.1.1.1)	1	Liver	1
Fumerase (*FUM*; E.C.4.2.1.2)	1	Muscle	3
Fructose diphosphatase (*FDP*; E.C.3.1.3.11)	1	Muscle	2
Glucoaminidase (*GA*)	2	Liver/Muscle	1
Glutamine dehydrogenase (*GDH*; E.C. 1.4.1.2)	1	Muscle	3
Glyceraldehyde-phosphate dehydrogease (*GAP*; E.C. 1.2.1.12)	2	Liver/Muscle	2
Glutamic-pyruvate transaminase (*GPT*; E.C.2.6.1.2)	2	Muscle	3
Hexokinase (*HK*; E.C.2.7.1.1)	1	Liver/Muscle	2
Isocitrate dehydrogenase (*IDH*; E.C.1.1.1.41)	2	Liver/Muscle	2
Lactate dehydrogenase (*LDH*; E.C.1.1.1.27)	2	Muscle	1
Leucine dehydrogenase (*LeuDH*)	2	Muscle	2
Monoamine oxidase (*MO*; E.C.1.4.3.4)	1	Muscle	4
Malic enzyme (*ME*; E.C.1.1.1.40)	2	Muscle	2
Methylumbelliferyl phosphatase (*MUP*;E.C.3.1.3.2)	2	Liver/Muscle	4
Nonspecific garget stain (*NSG*)	1	Muscle	1
Peptidase (*PEP*;E.C.3.4.11)	1	Muscle	3
Phosphoglucose isomerase (*PGI*; E.C.5.3.1.8)	1	Muscle	1
Phosphoglucomutase (*PGM*; E.C.2.7.5.1)	2	Muscle	1
Phosphomannose isomerase (*PMI*; E.C.5.3.1.8)	1	Liver	1
6-Phosphogluconate dehydrogenase (*6PG*; E.C.1.1.1.44)	1	Muscle	2
Superoxide dismutase (*SOD*; E.C.1.15.1.1)	1	Liver	1
Sorbitol dehydrogenase (*SDH*; E.C. 1.1.1.14)	1	Muscle	1
Triose phosphate isomerase (*TPI*; E.C.5.3.1.1)	1	Liver	2
Xanthine dehydrogenase (*XDH*; E.C.1.2.1.37)	1	Liver	2

[a] Electrophoretic buffers: (1) LiOH, pH 8.1 (Ridgway et al. 1970); (2) N-(3-aminopropyl)-morpholine, pH 6.1 (Clayton and Tretiak 1972); (3) Tris-borate-EDTA, pH 8.7 (Markert and Faulhaber 1965); (4) Tris-citrate-EDTA, pH 7.0 (Ayala et al. 1974). Electrode buffer 4 was diluted 1:15 for gel buffer.

Table 3. Allele frequencies of four polymorphic loci in Atlantic herring.

Sample		ACON-2 N	*100*	*95*	*86*	*105*	*60*	EST N	*100*	*96*	*104*	*98*	*102*	*94*
Spring spawners														
Carleton	1979	44	0.932	0.068	0.0	0.0	0.0	43	0.802	0.198	0.0	0.0	0.0	0.0
	1980	62	0.863	0.121	0.008	0.0	0.008	63	0.794	0.198	0.008	0.0	0.0	0.0
Ile Verte	1979	67	0.896	0.060	0.022	0.022	0.0	60	0.725	0.258	0.008	0.0	0.008	0.0
	1980	98	0.832	0.123	0.026	0.020	0.0	98	0.801	0.179	0.015	0.005	0.0	0.0
Fall spawners														
Jefferies Ledge	1978	81	0.840	0.154	0.006	0.0	0.0	79	0.633	0.348	0.013	0.0	0.0	0.006
	1979	89	0.758	0.219	0.223	0.0	0.0	104	0.668	0.313	0.014	0.005	0.0	0.0
	1980	96	0.813	0.172	0.100	0.005	0.0	100	0.690	0.300	0.005	0.0	0.0	0.005
Lurcher Shoals	1978	74	0.878	0.115	0.0	0.067	0.0	74	0.716	0.284	0.0	0.0	0.0	0.0
	1979	90	0.811	0.183	0.006	0.0	0.0	90	0.689	0.294	0.011	0.0	0.0	0.006
	1980	95	0.879	0.111	0.005	0.005	0.0	94	0.761	0.239	0.0	0.0	0.0	0.0
Miscou	1979	47	0.787	0.213	0.0	0.0	0.0	47	0.670	0.309	0.021	0.0	0.0	0.0
Prince Edward Island	1979	98	0.867	0.107	0.020	0.005	0.0	97	0.773	0.201	0.016	0.005	0.005	0.0
Stonehaven	1979	41	0.793	0.183	0.024	0.0	0.0	38	0.776	0.224	0.0	0.0	0.0	0.0
	1980	96	0.839	0.141	0.020	0.0	0.0	96	0.781	0.214	0.005	0.0	0.0	0.0

Sample		LDH-2 N	*100*	*72*	*124*	PGM-2 N	*100*	*92*	*112*	*82*	*95*
Spring spawners											
Carleton	1979	44	0.943	0.057	0.0	44	0.591	0.386	0.011	0.012	0.0
	1980	84	0.964	0.036	0.0	63	0.460	0.468	0.048	0.024	0.0
Ile Verte	1979	67	0.955	0.045	0.0	67	0.641	0.321	0.022	0.016	0.0
	1980	98	0.980	0.020	0.0	98	0.602	0.398	0.0	0.0	0.0
Fall spawners											
Jefferies Ledge	1978	81	0.969	0.031	0.0	81	0.691	0.284	0.012	0.006	0.006
	1979	104	0.947	0.053	0.0	104	0.548	0.414	0.038	0.0	0.0
	1980	100	0.945	0.055	0.0	94	0.500	0.431	0.021	0.048	0.0
Lurcher Shoals	1978	74	0.980	0.020	0.0	74	0.574	0.399	0.027	0.0	0.0
	1979	90	0.972	0.028	0.0	90	0.500	0.494	0.006	0.0	0.0
	1980	95	0.911	0.089	0.0	90	0.556	0.361	0.056	0.022	0.006
Miscou	1979	47	0.958	0.042	0.0	47	0.606	0.372	0.021	0.0	0.0
Prince Edward Island	1979	100	0.950	0.035	0.015	87	0.581	0.368	0.040	0.006	0.005
Stonehaven	1979	41	0.939	0.049	0.012	41	0.561	0.427	0.0	0.012	0.0
	1980	96	0.969	0.031	0.0	91	0.599	0.374	0.022	0.0	0.006

Table 4. Allelic frequencies of *Phosphoglucose isomerase-2* in Atlantic herring.

Spring spawners		N	*100*	*-75*	*-3*	*150*	*-115*	*40*	*120*	*140*	*210*	*-11*	*200*	*140 (null)*	*158*	*-118*
Carleton	1979	44	0.727	0.091	0.057	0.091	0.023	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1980	77	0.656	0.091	0.078	0.149	0.020	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.007	0.0
Ile Verte	1979	67	0.687	0.105	0.112	0.082	0.008	0.008	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1980	98	0.735	0.097	0.082	0.082	0.0	0.005	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fall spawners																
Jefferies Ledge	1978	81	0.670	0.136	0.074	0.093	0.006	0.006	0.006	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1979	104	0.630	0.125	0.082	0.111	0.029	0.005	0.005	0.005	0.005	0.0	0.0	0.0	0.0	0.00
	1980	99	0.657	0.121	0.111	0.106	0.0	0.0	0.0	0.0	0.0	0.005	0.0	0.0	0.0	0.0
Lurcher Shoals	1978	74	0.669	0.149	0.108	0.061	0.014	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1979	89	0.663	0.118	0.101	0.112	0.0	0.0	0.006	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1980	95	0.674	0.126	0.105	0.068	0.021	0.0	0.0	0.0	0.0	0.005	0.0	0.0	0.0	0.0
Miscou	1979	45	0.544	0.100	0.133	0.078	0.044	0.011	0.022	0.0	0.0	0.0	0.044	0.022	0.0	0.0
Prince Edward Island	1979	95	0.590	0.105	0.132	0.137	0.026	0.005	0.0	0.005	0.0	0.0	0.0	0.0	0.0	0.0
Stonehaven	1979	41	0.707	0.085	0.085	0.098	0.024	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1980	95	0.626	0.153	0.095	0.095	0.016	0.011	0.0	0.0	0.005	0.0	0.0	0.0	0.0	0.0

Table 5. Likelihood analysis of allelic frequencies in Atlantic herring.

Source of variation	df	*ACON-2*	df	*EST*	df	*LDH-2*	df	*PGI-2*	df	*PGM-2*	df	SUM
Between spring and fall spawners (total)	2	16.85**	1	9.33*	1	1.23	4	85.18**	2	2.88	10	115.47***
Among fall spawners (1978)	2	9.98*	1	2.42	1	0.35	4	2.61	2	4.69	10	20.05*
Between spring and fall (1979)	2	20.29**	1	1.75	1	0.05	4	5.97	2	3.72	10	31.77***
Among spring spawners	2	6.20	1	1.65	1	0.16	4	3.02	2	1.24	10	12.27
Among fall spawners	8	16.38	4	8.44	4	2.26	16	33.03*	8	15.08	40	75.18***
Between spring and fall (1980	2	3.61	1	3.52	1	5.18	4	6.47	2	9.96*	10	28.74**
Among spring spawners	2	2.27	1	0.03	1	0.79	4	7.07	2	14.97**	10	25.13**
Among fall spawners	4	3.71	2	4.65	2	6.00	8	7.64	4	8.68	20	30.68

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 6. Genetic similarities (I_N) between samples of Atlantic herring for *ACON-2*, *EST*, *PGI-2*, and *PGM-2*. Sample abbreviations in Table 1.

C80	0.993												
IV79	0.996	0.984											
IV80	0.997	0.992	0.994										
JL78	0.983	0.969	0.993	0.983									
JL79	0.985	0.987	0.986	0.989	0.989								
JL80	0.989	0.993	0.989	0.991	0.985	0.997							
LS78	0.994	0.989	0.996	0.993	0.990	0.993	0.995						
LS79	0.987	0.993	0.984	0.989	0.980	0.997	0.998	0.993					
LS80	0.997	0.993	0.996	0.996	0.987	0.991	0.994	0.997	0.991				
M79	0.982	0.980	0.987	0.984	0.990	0.995	0.991	0.991	0.989	0.989			
PEI79	0.995	0.993	0.994	0.994	0.983	0.988	0.990	0.994	0.988	0.997	0.990		
S79	0.991	0.991	0.987	0.995	0.979	0.991	0.992	0.989	0.992	0.991	0.986	0.990	
S80	0.995	0.992	0.995	0.997	0.987	0.992	0.993	0.996	0.990	0.998	0.991	0.998	0.992
	C79	C80	IV79	IV80	JL78	JL79	JL80	LS78	LS79	LS80	M79	PEI79	S79
	Spring spawners				Fall spawners								

Appendix. Allelic frequencies at marginally polymorphic loci in Atlantic herring.

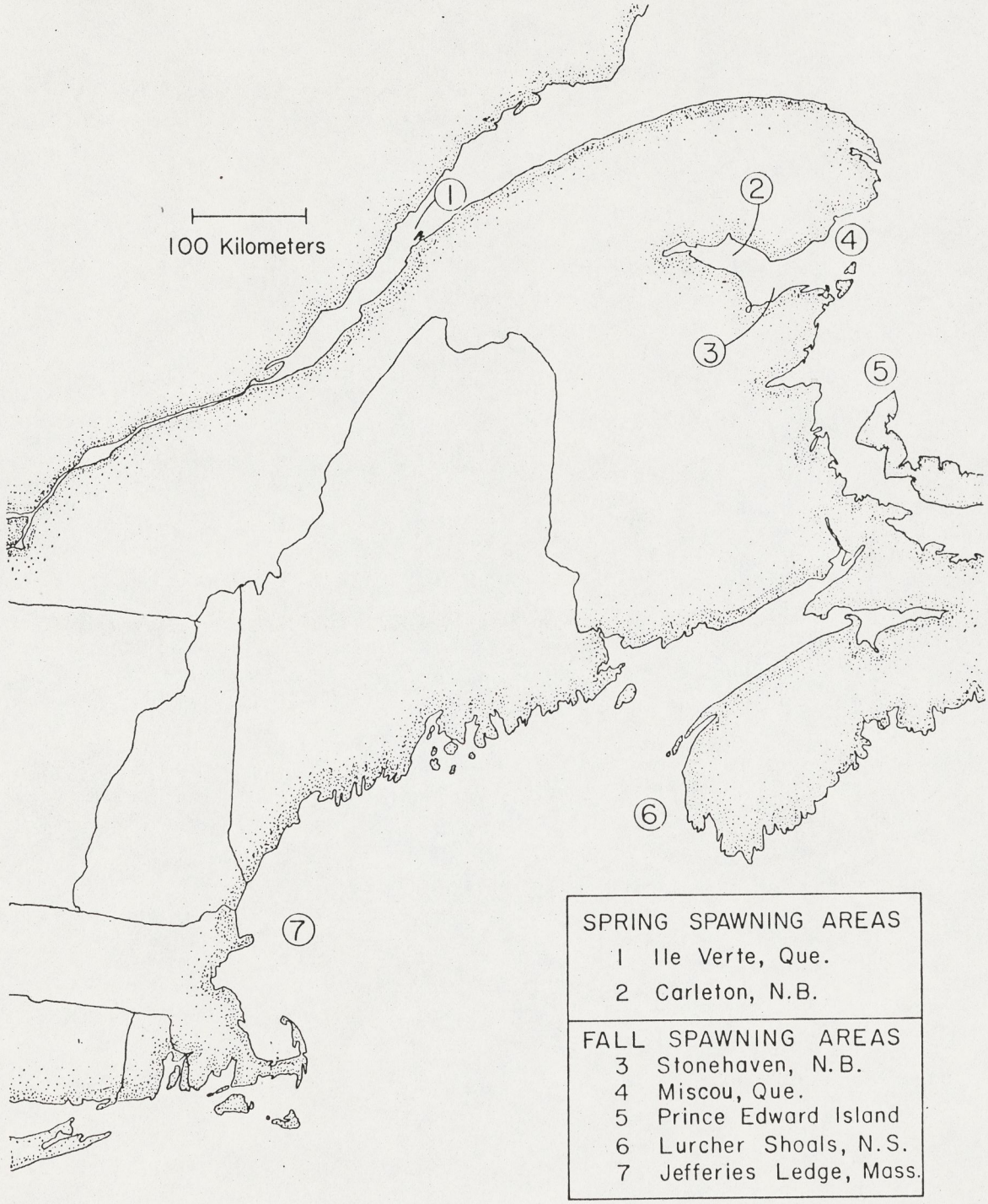
	AAT-1			*IDH-1*				*IDH-2*						
	N	*100*	*96*	*106*	N	*100*	*160*	*58*	N	*100*	*113*	*104*	*90*	*94*	*122*
C79	44	0.989	0.011	0.0	44	1.0	0.0	0.0	44	0.966	0.011	0.023	0.0	0.0	0.0
C80	84	0.994	0.0	0.006	84	1.0	0.0	0.0	84	0.976	0.0	0.0	0.024	0.0	0.0
IV79	67	0.992	0.998	0.0	67	1.0	0.0	0.0	67	0.970	0.008	0.015	0.008	0.0	0.0
IV80	98	0.990	0.010	0.0	98	1.0	0.0	0.0	98	0.980	0.010	0.005	0.005	0.0	0.0
JL78	81	0.988	0.012	0.0	81	0.994	0.006	0.0	81	0.982	0.018	0.0	0.0	0.0	0.0
JL79	104	0.986	0.010	0.004	104	0.990	0.0	0.010	104	0.986	0.014	0.0	0.0	0.0	0.0
JL80	100	0.990	0.010	0.0	100	1.0	0.0	0.0	90	0.944	0.022	0.022	0.006	0.006	0.0
LS78	74	0.993	0.007	0.0	74	1.0	0.0	0.0	74	0.993	0.0	0.007	0.0	0.0	0.0
LS79	90	0.994	0.006	0.0	90	1.0	0.0	0.0	93	0.962	0.022	0.005	0.005	0.0	0.005
LS80	95	0.974	0.021	0.005	95	1.0	0.0	0.0	95	0.984	0.0	0.011	0.005	0.0	0.0
M79	47	0.979	0.021	0.0	47	1.0	0.0	0.0	47	0.958	0.011	0.011	0.011	0.011	0.0
PEI79	98	0.995	0.005	0.0	98	0.995	0.005	0.0	98	0.990	0.010	0.0	0.0	0.0	0.0
S79	41	0.976	0.024	0.0	41	1.0	0.0	0.0	41	0.963	0.012	0.012	0.012	0.0	0.0
S80	96	0.990	0.010	0.0	96	1.0	0.0	0.0	96	0.990	0.005	0.005	0.0	0.0	0.0

	LDH-1			*ME-1*			*ME-2*						
	N	*0*	*100*	N	*100*	*200*	N	*100*	*35*	*92*	*71*	*121*	*55*
C79	44	1.0	0.0	44	1.0	0.0	44	1.0	0.0	0.0	0.0	0.0	0.0
C80	84	0.988	0.012	84	1.0	0.0	84	0.994	0.006	0.0	0.0	0.0	0.0
IV79	67	0.993	0.007	67	1.0	0.0	67	1.0	0.0	0.0	0.0	0.0	0.0
IV80	98	0.990	0.010	98	0.995	0.005	98	0.990	0.005	0.0	0.0	0.005	0.0
JL78	81	0.994	0.006	81	1.0	0.0	81	0.982	0.006	0.0	0.006	0.006	0.0
JL79	104	0.986	0.014	104	1.0	0.0	104	0.990	0.010	0.0	0.0	0.0	0.0
JL80	100	0.990	0.010	100	1.0	0.0	100	0.980	0.010	0.005	0.005	0.0	0.0
LS78	74	0.993	0.007	74	1.0	0.0	74	0.993	0.007	0.0	0.0	0.0	0.0
LS79	90	0.989	0.011	90	1.0	0.0	90	0.982	0.006	0.0	0.006	0.006	0.0
LS80	95	0.995	0.005	95	1.0	0.0	95	0.974	0.0	0.016	0.010	0.0	0.0
M79	47	0.989	0.011	47	1.0	0.0	47	0.947	0.0	0.043	0.0	0.0	0.010
PEI79	98	0.995	0.005	98	1.0	0.0	98	0.995	0.005	0.0	0.0	0.0	0.0
S79	41	0.988	0.012	41	1.0	0.0	41	0.963	0.0	0.037	0.0	0.0	0.0
S80	96	0.974	0.026	96	1.0	0.0	96	0.969	0.0	0.031	0.0	0.0	0.0

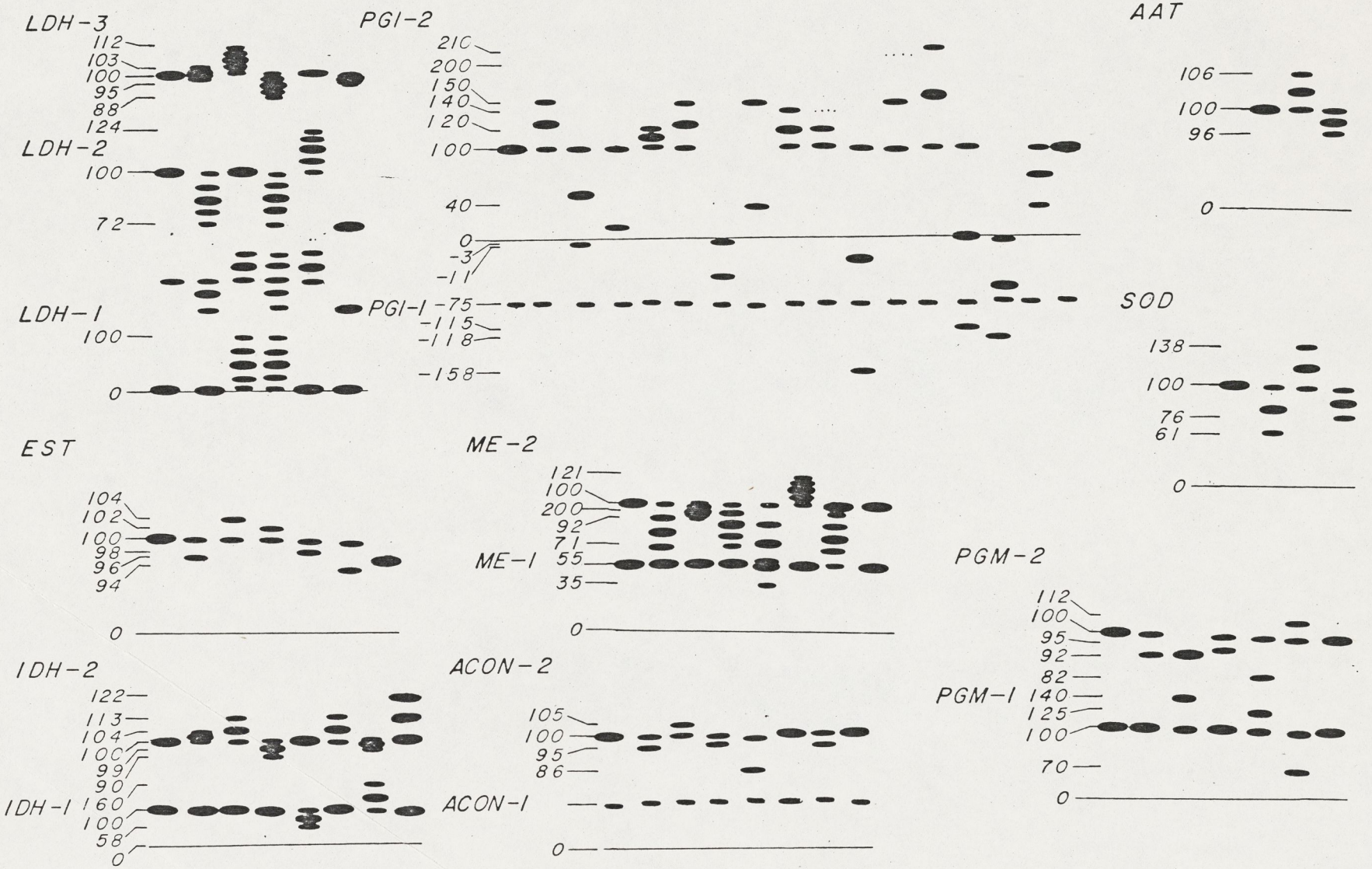
	PGM-1					SOD			
	N	*100*	*125*	*140*	*70*	N	*100*	*138*	*61*
C79	44	0.966	0.034	0.0	0.0	44	0.943	0.034	0.023
C80	84	0.958	0.024	0.0	0.018	84	0.970	0.0	0.030
IV79	67	0.955	0.030	0.008	0.008	67	0.993	0.007	0.0
IV80	98	0.954	0.041	0.005	0.0	98	0.995	0.005	0.0
JL78	81	0.975	0.025	0.0	0.0	76	1.0	0.0	0.0
JL79	103	0.985	0.010	0.005	0.0	104	0.990	0.005	0.005
JL80	100	0.950	0.015	0.035	0.0	100	1.0	0.0	0.0
LS78	74	0.980	0.020	0.0	0.0	74	1.0	0.0	0.0
LS79	90	0.967	0.028	0.0	0.005	90	0.994	0.006	0.0
LS80	95	0.984	0.011	0.0	0.005	95	1.0	0.0	0.0
M79	47	0.979	0.021	0.0	0.0	47	1.0	0.0	0.0
PEI79	90	0.972	0.017	0.011	0.0	98	0.995	0.005	0.0
S79	41	0.951	0.024	0.012	0.012	41	0.988	0.012	0.0
S80	96	0.969	0.016	0.016	0.0	96	1.0	0.0	0.0

Figure 1. Sampling locations of Atlantic herring from the Gulf of St. Lawrence and the Gulf of Maine.

Figure 2. Observed electrophoretic phenotypes for all variable loci in Atlantic herring. See text for explanation.



SPRING SPAWNING AREAS	
1	Ile Verte, Que.
2	Carleton, N.B.
FALL SPAWNING AREAS	
3	Stonehaven, N.B.
4	Miscou, Que.
5	Prince Edward Island
6	Lurcher Shoals, N.S.
7	Jefferies Ledge, Mass.


LDH-3
112
103
100
95
88
124
LDH-2
100
72
LDH-1
100
0
PGI-2
210
200
150
140
120
100
40
0
-3
-11
PGI-1 -75
-115
-118
-158
AAT
106
100
96
0
SOD
138
100
76
61
0
EST
104
102
100
98
96
94
0
ME-2
121
100
200
92
71
ME-1 55
35
0
PGM-2
112
100
95
92
82
PGM-1 140
125
100
70
0
IDH-2
122
113
104
100
99
90
IDH-1 160
100
58
0
ACON-2
105
100
95
86
ACON-1
0

Templeton, A.R. 1980. Modes of speciation and inferences based on genetic distances. Evol. 34(4): 719-729.

- Founders Princ. - genet. revolution - not revol. but Genetic transilience - only few loci change - not majority
- Genetic Distance messures largely from enzyme coding loci - which ~~are~~ appear to be neutral in respect to transilience + circumstantial - evidence that enzyme-coding loci are relatively insensitive markers of speciation and macroevolutionary events in general (- - -) alleles not involved w/ speciation

UNIVERSITY OF MAINE *at Orono*

Department of Zoology

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Orono, Maine 04469
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September 21, 1981

Dr. Robert Behnke
Department of Fisheries and
Wildlife Biology
Colorado State University
Fort Collins, CO 80523

Dear Bob

I am being considered for tenure this year at the University - it will be my fifth year as an assistant professor. The review committee requests that I solicit letters from professional colleagues who can evaluate my work and my potential. It's a large favor to ask, but I'd very much appreciate it if you could write a letter in my behalf. I feel that you are familiar with some of my research and the field in general so that you could accurately appraise my contributions and future potential. In addition to the work with which you are familiar, I've enclosed copies of my other publications.

I've continued my cichlid work which I presented last year in my seminar at CSU, and have recently had a manuscript on some of the results accepted by Evolution. The story of the Cuatro Cienegas cichlids is still evolving, however, and the breeding experiments now underway will prove to be crucial.

I sincerely appreciate your effort in writing a letter of evaluation for me. Please submit it directly to:

Dr. William Valleau, Chairman
Department of Zoology
100 Murray Hall
University of Maine
Orono, ME 04469
U.S.A.

In the event that you do not feel qualified to compose such an evaluation or if you believe that you would not be able to write the letter in the very near future, please also communicate that directly to Dr. Valleau. Again, thanks very much for your continued assistance.

Best regards,

Irv Kornfield
Assistant Professor

IK/pab
encl.

Evolution, 33(1), 1979, pp. 1–14

BIOCHEMICAL AND CYTOLOGICAL DIFFERENTIATION AMONG CICHLID FISHES OF THE SEA OF GALILEE

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Among vertebrates, fishes of the family Cichlidae stand out as an evolutionary success. Cichlids are diverse ecologically and dominate the ichthyofauna of Africa and tropical America. Some species with broad ecological tolerances are widely distributed, while other more specialized forms have very restricted distributions. Members of endemic species flocks may exhibit complex taxonomic relationships because of multiple episodes of ecological convergence. In the great lakes of Africa, high rates of speciation are characteristic of some genera within species flocks (Greenwood, 1974; Kornfield, 1978). Although novel modes of speciation have been repeatedly postulated to explain this apparent diversity (see Fryer and Iles, 1972; Fryer, 1977), the genetic changes associated with cladogenesis have not been studied in a systematic manner.

Descriptive cytological studies have indicated that there is limited variation in chromosome number and morphology among Old World species (Jakowska, 1950; Jalabert et al., 1971; Natarajan and Subrahmanyam, 1968; Post, 1965; Thompson, 1976). Similarly, little interspecific variation has been noted for quantitative estimates of nuclear DNA (Hinegardner and Rosen, 1972). However, in order to appreciate the evolutionary significance of apparently limited genetic modification, the data must be evaluated against a rigid systematic framework. Only then can genetic modifications associated with speciation be distinguishable from continuous phyletic change.

To characterize evolutionary changes in the Cichlidae, we have investigated fishes at the northern limit of the family's Old World range in Israel. The six native cichlids are sympatric in the Sea of Galilee. An additional species in coastal rivers, *Sarotherodon niloticus*, was recently eliminated by pollution (Goren, 1974). Previous studies of the Israeli cichlid fauna have considered morphology, ecology, reproductive biology, and zoogeography (see references in Steinitz, 1954; Werner, 1976). Three of the extant species are widespread over North Africa. *Sarotherodon aureus* (Steindachner, 1864) ranges from Nigeria into Israel, *S. galilaeus* (Artedi, 1757) ranges from Liberia and Senegal, and *Tilapia zillii* (Gervais, 1848) ranges south to Uganda. Two additional species belong to an endemic Middle Eastern genus: *Tristramella simonis* (Günther, 1864) occurs in the Sea of Gal-

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Likely pre-Suez occurrence of a Red Sea fish *Aphanius dispar* in the Mediterranean

THE construction of artificial waterways connecting different faunal provinces provides biologists with an opportunity to observe ecological, biogeographic and evolutionary changes. But recognition of change requires a thorough knowledge of conditions before construction. The opening of the Suez Canal in 1857 is a case in point. More than thirty species of Red Sea fishes have been recorded as colonising the Mediterranean since the opening of the canal[1], but because no reliable systematic ichthyological collections were made in the eastern Mediterranean before 1857, these new records must be judged with caution. We report here comparative electrophoretic evidence which suggests that the Red Sea cyprinodontid, *Aphanius dispar*, first reported along the Israeli coast in 1947 (ref. 2) and therefore considered a Suez migrant, has been a permanent Mediterranean resident for a long time.

A. dispar (Rüppell) is a small, littoral, euryhaline fish widely distributed in the Indian Ocean, which also occurs along both coasts of the Sinai Peninsula and in the Suez Canal. In the Mediterranean, it was reported at Port Said[3] and subsequently along the Israeli coast from Atlit, Tel Aviv, and Caesarea[2,4]. In addition, several subspecifically distinct populations (*A. dispar richardsoni* (Boulenger)) occur as isolates in freshwater pools along the periphery of the Dead Sea. These Dead Sea fishes have been living in isolation since the early Pleistocene[5] and differ from conspecifics in male breeding coloration[8]. But all populations of the species have the same gross karyotype, cross freely in aquaria and are morphologically indistinguishable[6,7].

We collected population samples of *A. dispar* at five localities from the Mediterranean, Red Sea and Dead Sea (Fig. 1). All specimens were examined for genetic variability at nineteen putative isoenzyme loci by standard methods of starch gel electrophoresis[8] (Table 1). Genetic similarity between all possible pairs of these populations was calculated by means of Rogers' coefficient (S_R); the values are provided in Table 2.

In general, excluding comparisons between subspecies and karyotypically differentiated populations (which could easily be regarded as sibling species), virtually all intraspecific estimates of genetic similarity (S_R) in a wide variety of organisms have been greater than 0.75[10,11]. As expected, both population pairs within the Red Sea and Dead Sea exhibit a very high degree of similarity. The estimates of similarity for *A. dispar* between the Mediterranean, Red Sea and Dead Sea are in sharp contrast, however (Table 2). Because *A. dispar* was presumed to have entered the Mediterranean only recently through the Suez Canal[2,12], it was

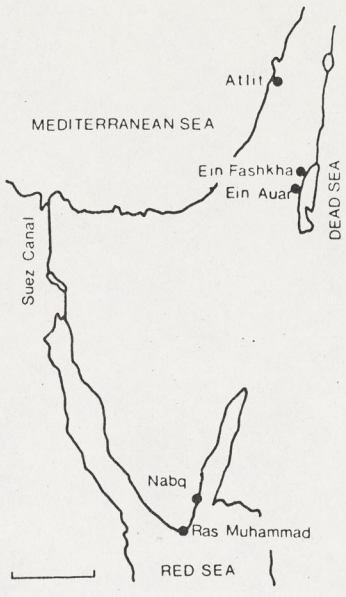

Fig. 1 Sampling localities of *Aphanius dispar* in Israel. The scale equals 100 km.

expected that this population sample would approximate the level of divergence observed among Red Sea samples. Instead, the degree of difference between Mediterranean and Red Sea samples is as large as that between either sample and the Dead Sea isolates, whose last conspecific contact was during the early Pleistocene. Such genetic differences are of the order normally associated with interspecific comparisons, where long periods of isolation are indicated[13]. We conclude therefore that *Aphanius dispar* was extant in the Mediterranean before the construction of the Suez Canal.

Our conclusion reinforces speculations about the pre-Suez occurrence of *A. dispar* in the Mediterranean. This species has previously[14,15] been considered a likely candidate for pre-Suez migration because of its wide salinity tolerance and the high probability of early interoceanic contact facilitated by ancient channelling[16] and eustatic fluctuations.

At least two alternative explanations for our observations, consistent with the idea of recent Suez migration, merit attention. First, stochastic events associated with colonisation may have reduced the level of variability and provided a biased sample of founding Red Sea genes. Although a normal level of heterozygosity could easily become re-established if population size increased rapidly, the

Table 1 Genetic variation at 19 loci in five populations of *A. dispar*

Sample area	Locality	Heterozygosity*	Average no. of alleles per locus	Unique alleles†	
Mediterranean	Atlit	6.27	1.26	6	
Red Sea	Nabq	2.39	1.37	8	
Red Sea	Ras Muhammad	6.00	1.26		
Dead Sea	Ein Fashkha	5.10	1.26	6	
Dead Sea	Ein el Ghuweir	4.56	1.11		

Thirty individuals were examined from each locality. With the exception of the assay for lactate dehydrogenase (LDH), all proteins were examined from whole-body homogenates diluted 1:1 with deionised water. LDH was examined from individual homogenates of eye. Electrophoretic procedures and histochemical staining were similar to those described before[8]. Specimens were examined for the following proteins (number of presumed loci in parentheses): esterase (2), LDH (3), malate dehydrogenase (2), phosphoglucomutase (3), isocitrate dehydrogenase (1), general protein (2), α-glycerophosphate dehydrogenase (1), 6-phosphogluconate dehydrogenase (1), phosphoglucose isomerase (2), aldolase (1), and amino peptidase (1).

*Heterozygosity was calculated as the average observed number of heterozygotes per locus, expressed ($\times 100$).

†Unique alleles are those alleles which were observed in only one out of the three sample areas.