A sympatric sibling species complex of *Petrotilapia* Trewavas from Lake Malawi analysed by enzyme electrophoresis (Pisces: Cichlidae)

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The present electrophoretic data for *Petrotilapia*, referred to earlier works on sibling species, were examined by standard electrophoresis. The results indicated that, for the three taxa, no alternative fixed alleles were found in any of the 25 loci examined. However, having more gene frequencies were indicated at seven polymorphic loci. This suggests that either the taxa are related sibling species, which results, diverged so that they are "incipient" species with minimal gene flow between them. The genetic and field data lend support to the hypothesis that sympatric splitting of morphs could be important in the explosive radiation of the cichlids.

**KEYWORDS:** Evolutionary speciation, polymorphism, enzymes, genetics, fish electrophoresis, morph selection, cichlid.

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**INTRODUCTION**

The species flocks of cichlid fishes within the Rift Valley Lakes of Africa represent a classic case of explosive speciation [Frey & Hess, 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. Riblhin, pers. comm.; K. McKay, pers. obs.). Despite the overwhelming evidence of diversity among cichlids of all three of the large great lakes of Africa, an electrophoretic study of a phenomorphically polymorphic Mexican cichlid (Sage & Selander, 1979) has been used to cast doubt upon whether or not the African cichlid species are really as species-rich as reported (Kirkpatrick & Selander, 1979; Graven & Rosenblatt, 1980). Sage & Selander (1973) found that three distinct cichlid morphotypes from lakes in Cuatro Cienegas region of Mexico, which have been treated as an endemic species flock (Kornfield & Koch, 1975), could not be distinguished electrophoretically. Their results suggest that the morphs represent a single polymorphic species. Sage & Selander (1979), 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 be groups of sibling species (Holdenberg, 1978; Schröder, 1980; Marsh et al., 1981). These studies on two of the Lake Malawi cichlids, Pseudotropheus zebra Boulenger and Pseudotropheus nigrofasciatus Trewavas, suggest that sympatric morphs separate into different gene pools on the basis of assortative mating by colour morphs. Pseudotropheus nigrofasciatus, which has been considered a single polymorphic species (Fryer, 1959), was divided into three sibling species, referred to by Marsh et al. (1981) as Big Blue BB, Orange 510 Kitten OC, and Orange Lappet OL. Field observations of non-interbreeding among colour morphs underline the suggestion by Marsh et al. (1981) that the three are distinct sibling species. This conclusion is reached in spite 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; McKay, 1976b, 1980) led us to examine enzyme polymorphisms within these three 'sibling' species of P. nigrofasciatus. The two questions which we wished to answer were: are different, alternative alleles fixed at any locus for the three taxa, and are allelomorph frequencies homogenous or heterogeneous among taxa? Reproductive isolation between sympatric species would be demonstrated unambiguously if alternative alleles are fixed at an enzyme locus (Ayala & Powell, 1972). Heterogeneous gene frequencies among colour morphs would suggest that these taxa are not interbreeding randomly. Homogenous frequencies among colour morphs would suggest either a high degree of interbreeding among the groups, or parallel variation at the loci tested.

METHODS

Protein electrophorons for the three taxa were examined using standard horizontal starch-gel electrophoresis and histochemical staining procedures (Selander et al., 1971; Harrison, 1978; Allen et al. 1978). 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 two females of OC, 19 males and one female of OL, and 13 males and nine females of BB.
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 dithiothreitol and 0.3% polyvinylpyrrolidone (PVP) at -196°C in liquid nitrogen for 4 weeks. The samples were transferred to a freezer at -60°C on return to the laboratory in the U.S.A. and remained there 8 months until analysis.

RESULTS

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

Eighteen of the 25 loci studied did not vary in our survey: Esterase-1, General protein-1, General protein-3, 6-phosphogluconate dehydrogenase, X-glycerophosphate dehydrogenase, Hexokinase, Isocitrate dehydrogenase, Glutamate oxaloacetate transaminase, Malic enzyme, Malate dehydrogenase, Lactate dehydrogenase, Alcohol dehydrogenase, Peptidase-2, Glucose-6-phosphate dehydrogenase, Phosphoglucomutase isomerase-1, Phosphoglucomutase isomerase-2, Phosphoglucomutase mutase-1 and Superoxide dismutase. Seven putative loci were found to be polymorphic: Esterase-1, Esterase-2, Esterase-3, Esterase-4, Peptidase-1 (Pep-1), Peptidase-2 (Pep-2), Cerebral kinase (CK), Phosphoglucomutase mutase-3 (PGM-3) and Succinic dehydrogenase.

Within population samples, the polymorphic loci were all found to be in Hardy-Weinberg equilibrium, and there were no significant differences in gene frequencies between the sexes. None of the loci examined were fixed for alternative alleles. However, allele frequencies were heterogeneous among taxa, there being significant differences from random expectation for all seven loci: G test, Table 1. For example, for CK the frequency of the α allele was 0.45 for OC, 0.33 for OL, and 0.80 for BB. For Pep-1, the frequency of the a allele was 0.05 for OC and BB, but 0.25 for OL. 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 alternative alleles at any of the loci we examined. Therefore, we found no clear diagnostic electromorph character to separate and identify these taxa. The results obtained are consistent with the ‘sibling’ species hypothesis. Marsh et al., 1981, or the polymorphic hypothesis. Fryer, 1939. Because electrophoretic divergences are time dependent (Carmicci et al., 1981), 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 colour morphs of P. tilapia, 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: either these forms represent isolated and differentiated gene pools, or ‘incipient’ species with minimal gene flow between morphs. Whether or not these taxa are newly evolved,
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*P<0.05, **P<0.01, G-test.

totally differentiated "sibling" species or 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 colour morphs. The study by Marsh et al. (1981) of P. tridentiger adds further evidence that coloration is important in species recognition or in mate selection within a species (Noble & Corrie, 1939; Fryer, 1950, 1977; Greenwood, 1974; McKay & Barlow, 1976; Eves & Lewis, 1979).

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 (Kruiswig, 1947, 1963; Lowe-McConnell, 1959; Trexavas et al., 1972; Barlow & Mumsey, 1976; McKay, 1978, 1980; Hoelzberg, 1970; Schneider, 1980). The heterogeneity in allelic frequencies demonstrated here among sympatric taxa parallels other studies in suggesting initial genetic isolation and differentiation occurs without geographical isolation. For example, another polychromatic Malawi cichlid, *Pseudotropheus zebra*, also has sympatric colour morphs that mate assortatively and that form two isolated breeding groups (Hoelzberg, 1970). Electrophoretic data on the four sympatric colour morphs of *P. zebra* show...
significant differences in allelic frequencies at four polymorphic loci (McKay et al., in prep.) which are consistent with the mating groups determined by Holzberg (1978). Holzberg (1978), and Schröder (1980) both speculate that the differentiated forms of P. zebra could have arisen sympatrically without geographical 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 Cichilidae is consistent with the three main developmental stages of the sympatric speciation model: 1. polymorphisms without assortative mating; 2. incomplete assortative mating and habitat separation by sympatric morphs; 3. complete assortative mating such that the morphs have achieved specific status. Maynard Smith's (1966) model, pers. comm. Furthermore, sympatric speciation by means of an intrinsic behavioral mechanism should allow more rapid splitting than does allopatric differentiation, which involves initial geographical separation followed by migration. Sympatric differentiation may thus be the most consistent and parsimonious hypothesis to explain some of the cichlid radiations 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 (1971) 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".

ACKNOWLEDGMENTS

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