Cour. Forsch.-Inst. Senckenberg, **94:** 55–62. – Frankfurt a. M., 1. 7. 1987

# Karyotype and isozyme patterns of five species of *Aulonocara* REGAN, 1922

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With 2 plates and 8 figures.

## Introduction

Simplified techniques for the study of fish chromosomes led to an increase in the knowledge of chromosomal evolution of fishes. Today, the chromosome complements of about 1000 fish species are known.

Some years ago the cichlids from Lake Malawi became economically important to the aquarium industry. A lot of new taxa and morphs were detected.

Only few are cytogenetically investigated, THOMPSON, 1976; MEYER & FOERSTER, 1984; MEYER & SCHARTL, 1984. In the literature the chromosome number of African cichlids is varying from 2n = 40 to 46.

The chromosome numbers of five taxa of *Aulonocara*, including eight populations are given.

Analysis of isozyme patterns has proven to be a useful tool in the estimation of phylogenetic relationships in many fish taxa. However, little use of isozyme analysis is made so far in cichlid fish. We therefore undertook a first approach to evaluate this method during our studies on the systematics of *Aulonocara*.

Two isozyme patterns, Lactatdehydrogenase and Esterase, are studied of five taxa of *Aulonocara*, including eight populations.

## Material and methods

### Chromosome preparations

The specimens were injected with 0.03 ml of a 0.01% solution of colchicine for about 4 hours, then killed and the gills from one side removed. After hypotonic treatment (0.5% KCl for 20 min) the gill epithelium was fixed in acetic alcohol (1: 3) and stained with Giemsa (5 to 10 min).

### Isozyme patterns

1) Lactatdehydrogenase (LDH; E.C. 1.1.1. 27) and 2) Esterases (EST). LDH-patterns were analyzed according to DAVIS (1964), EST-patterns were analyzed according to WILLIAMS & REISFELD (1964).

Samples were taken from individual fish by cutting off the dorsal fin. The tissue was homogenized in 0.1 M Tris-HCl (pH 8.0), centrifuged at 20000 x g, and the clarified supernatants were processed for electrophoresis.

This method proved to have the advantage that repeated analysis of different isozymes from the same fish may be performed, which is especially useful, if only a limited number of animals from a taxon in question are available. However, in some cases, fin tissue shows only a primitive pattern of some enzymes compared to the more complex patterns obtained usually from liver or eye homogenates.

## Results

### Chromosome analysis

The cytogenetic findings of this investigation are presented below. The chromosomes were arranged according to size and the position of the centromere.A metaphase and a karyotype of each population are presented in plate 1, figs. 1 to 4 and plate 2, figs. 5 to 8.

The chromosome number for the five taxa, including eight populations (MEYER et al., this issue), was found to be 2n = 44.

The majority of chromosomes were acrocentric on an average of 32. With the exception of one pair (second position in all karyotypes), the acrocentric chromosomes were not distinguishable from each other. Submetacentric chromosomes were found with an average of three pairs. One pair (first position in all karyotypes) was characteristic for all *Aulonocara*. 4 to 6 (average 4) metacentric chromosomes could be observed. A differentiation was not possible, heteromorphic sex chromosomes were not detectable.

The difficulty in studying fish chromosomes arises from their small size and their large number. The chromosomes of *Aulonocara* were between 2 and 5 µm in length.

### Material investigated

*Aulonocara baenschi* (Chipoka): specimens examined, 1 male and 1 female; metaphases counted, male = 5, female = 8; chromosome number 2n = 44, acrocentrics = 30, submetacentrics = 8, metacentrics = 6.

*Aulonocara baenschi* (Maleri Island): specimens examined, 1 male and 1 female; metaphases counted, male = 12, female = 0; chromosome number 2n = 44 acrocentrics = 30, submetacentrics = 8, metacentrics = 6.

*Aulonocara baenschi* (Nkhomo): specimens examined, 1 male and 1 female; metaphases counted, male = 6, female = 4; chromosome number 2n = 44, acrocentrics = 32, submetacentrics = 6, metacentrics = 6.

*Aulonocara korneliae* (Chisumulu Island): specimens examined, 1 male and 1 female; metaphases counted, male = 10, female = 5; chromosome number 2n = 44, acrocentrics = 32, submetacentrics = 8, metacentrics = 4.

*Aulonocara hueseri* (Likoma Island): specimens examined, 1 male and 1 female; metaphases counted, male = 11, female = 12; chromosome number 2n = 44, acrocentrics = 34, submetacentrics = 6, metacentrics = 4.

*Aulonocara* spec. (Likoma Island): specimens examined, 1 male and 1 female; metaphases counted, male = 7, female = 6; chromosome number 2n = 44, acrocentrics = 32, submetacentrics = 6, metacentrics = 6.

*Aulonocara stuartgranti* (Chilumba):specimens examined, 1 male and 1 female; metaphases counted, male = 0, female = 8; chromosome number 2n = 44, acrocentrics = 34, submetacentrics = 6, metacentrics = 4.

*Aulonocara stuartgranti* (Mbenji Island): specimens examined, 1 male and 1 female; metaphases counted, male = 13, female = 0; chromosome number 2n = 44, acrocentrics = 32, submetacentrics = 6, metacentrics = 6.

## Analysis isozyme patterns

The LDH pattern was uniform in all animals (males and females) tested from *A. baenschi* MEYER & RIEHL, 1985 (three populations); *A. kornellae, A. hueseri* and *A.* spec., all MEYER, RIEHL and ZETZSCHE (this issue); and *A. stuartgranti* MEYER & RIEHL, 1985 (two populations). Only the prominent B4-LDH isozyme was visible.

The EST pattern was more complex. In *A. baenschi, A. hueseri, A.* spec. and *A. stuartgranti* (males as well females) constantly 6 bands (RF values: 2.2; 3.0; 3.4; 3.6; 4.0 and 5.2) were visible with no variation amongst the different *Aulonocara* taxa and populations.

In *Aulonocara korneliae* the pattern differed from all the others and an interpopulational variation between male and female was noted. The RF-value were: Male: 2.0; 2.9; 3.1; 3.8; 4.2 and 5.2. Female: 2.2; 3.3; 4.3; 4.9 and 5.2.

This preliminary studies showed that isozyme analysis might be a useful tool in cichlid systematics. However, like in all other studies performed so far, a large number of enzymes and individuals have to be analyzed before a taxonomical evaluation of genetic distance can be done.

## Summary

The karyotypes and the isozyme patterns (LDH and EST) from *Aulonocara baenschi* (three populations), *Aulonocara korneliae*, *Aulonocara hueseri*, *Aulonocara* spec. and *Aulonocara stuartgranti* (two populations) were described and discussed.

The chromosome number was constantly 2n= 44. The chromosomes varied clearly in length and position of the centromere. In the present study, *Aulonocara* was found to have 4 to 6 metacentric, 4 to 8 submetacentric and 30 to 34 acrocentric chromosomes.

It was not possible to make differences in the chromosome complements of the five taxa. Heteromorphic gonosomes were not observed.

The LDH pattern was uniform in all the species (females and males). The EST pattern was more complex. The pattern of *Aulonocara korneliae* differed from all the others and a variation between males and females was observed too.

## References

DAVIS, B.J. (1964): Disc-Electrophoresis-II. - Ann. N.Y. Acad. Sci. 121: 404-427.

MEYER, M.K. & W. FOERSTER (1984): Un nouveau *Pseudotropheus* du lac Malawi avec des remarques sur le complexe *Pseudotropheus, Melanochromis* (Pisces, Perciformes, Cichlidae). - Revue fr. Aquariol. 10 (4): 107-112.

MEYER, M.K., R. RIEHL & H. ZETZSCHE (1987): A revision of the cichlid fishes of the genus *Aulonocara* REGAN, 1922 from Lake Malawi, with descriptons of six new species. - Cour. Forsch.-Inst. Senckenberg (this issue).

MEYER, M.K. & M. SCHARTL (1984): *Pseudotropheus (Mayiandia) hajomaylandi* n.sp. a new taxon from Lake Malawi. - Revue fr. Aquariol. 11 (2): 51-56.

THOMPSON, K.W. (1976): Some aspects of chromosomal evolution of the Cichlidae (Teleostei: Perciformes) with emphasis on neotropical fish. - Ph. D. Thesis, Univ. of Texas: 132 pp.

WILLIAMS, D.E & R.A. REISFELD (1964): Discelectrophoresis in polyacrylamide gels: extension to new conditions of pH and buffer. - Ann. N.Y. Acad. Sci. 121: 373-381.

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## Plate 1

Fig. 1. Metaphase (a) and karyotype (b) of a male of *Aulonocara baenschi* (Chipoka). Fig. 2. Metaphase (a) and karyotype (b) of a male of *Aulonocara baenschi* (Maleri Island). Fig. 3. Metaphase (a) and karyotype (b) of a male of *Aulonocara baenschi* (Nkhomo). Fig. Fig. 4. Metaphase (a) and karyotype (b) of a male of *Aulonocara korneliae* (Chisumulu Island).

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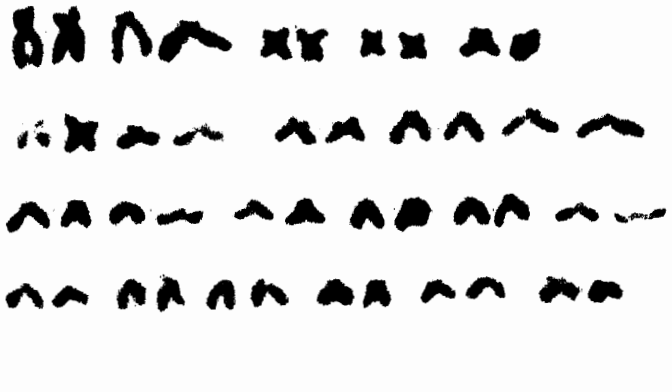

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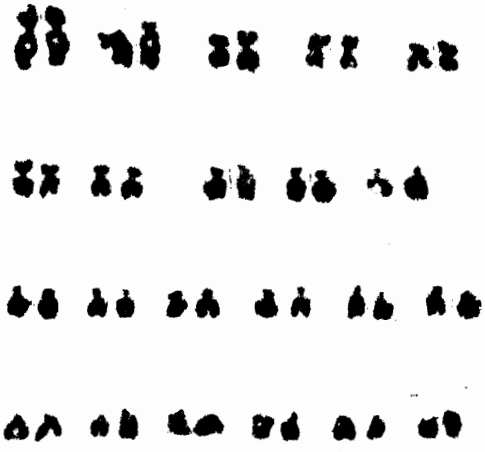

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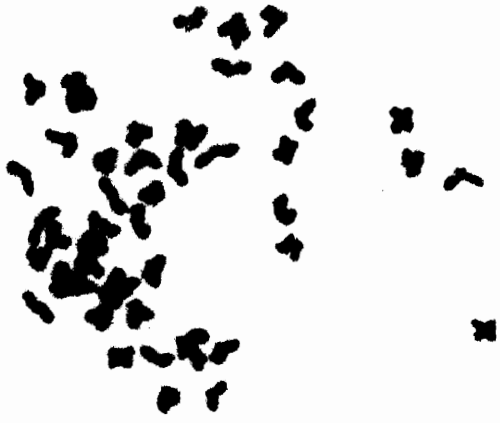

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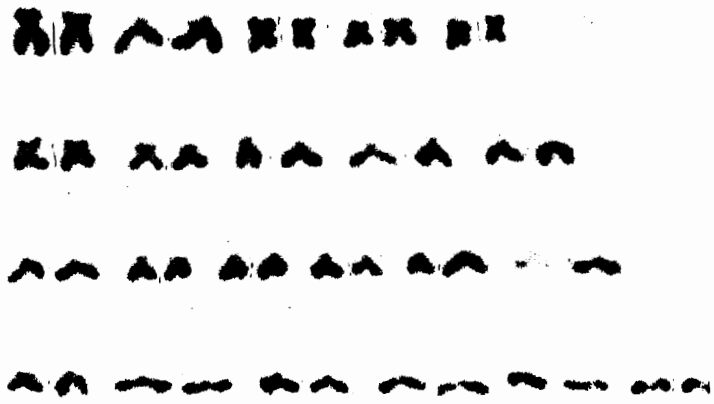

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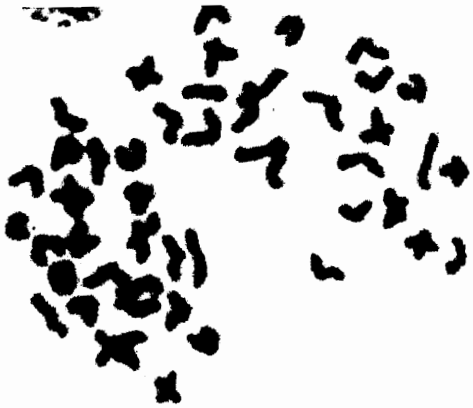

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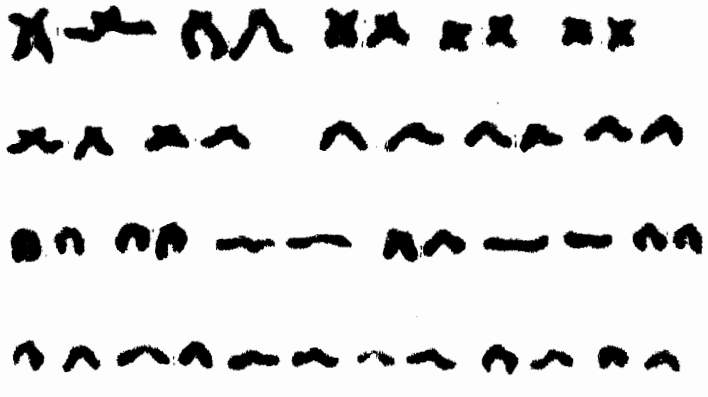
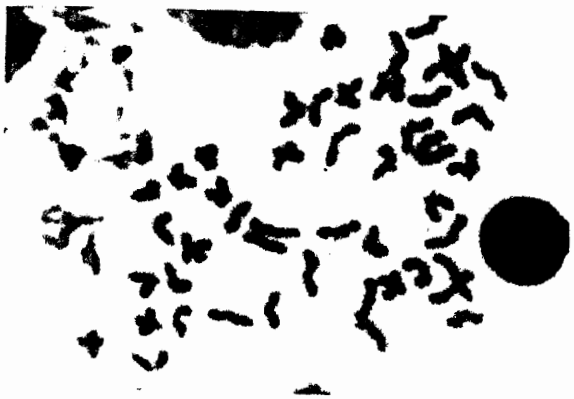


4b

## Plate 2

Fig. 5. Metaphase (a) and karyotype (b) of a male of *Aulonocara hueseri* (Likoma Island). Fig. 6. Metaphase (a) and karyotype (b) of a male of *Aulonocara* spec. (Likoma Island). Fig. 7. Metaphase (a) and karyotype (b) of a male of *Aulonocara stuartgranti* (Chilumba). Fig. 8. Metaphase (a) and karyotype (b) of a male of *Aulonocara stuartgranti* (Mbenji Island).



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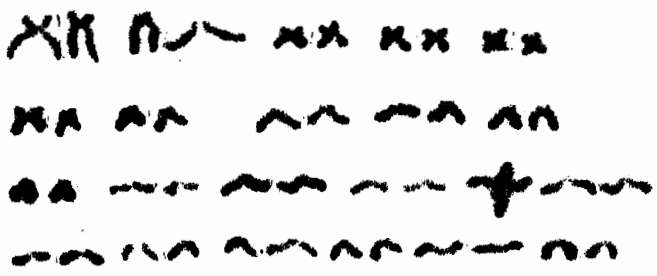

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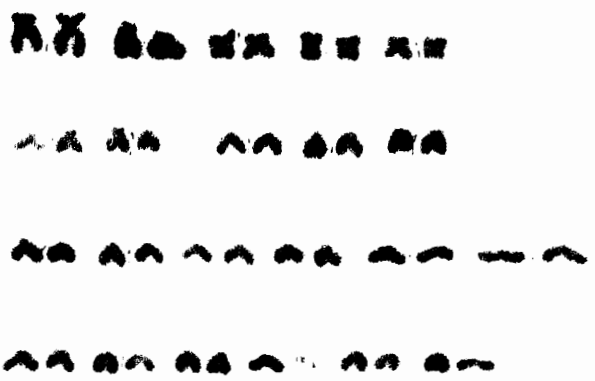

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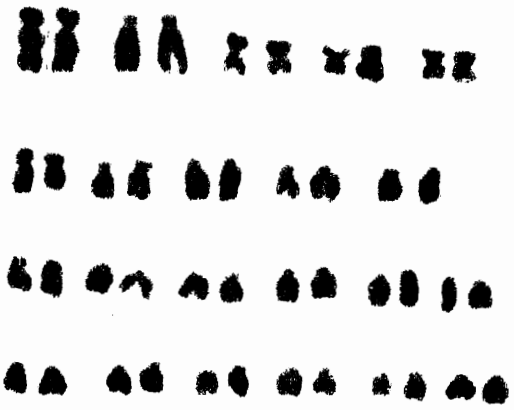

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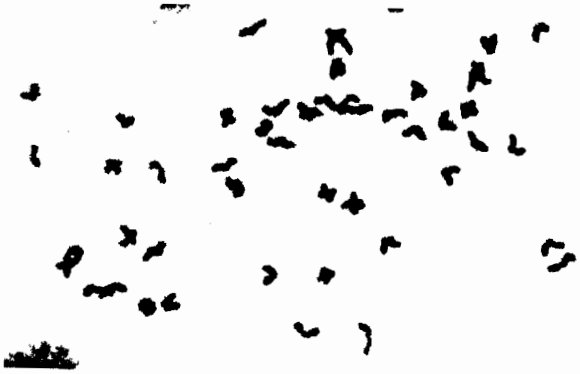

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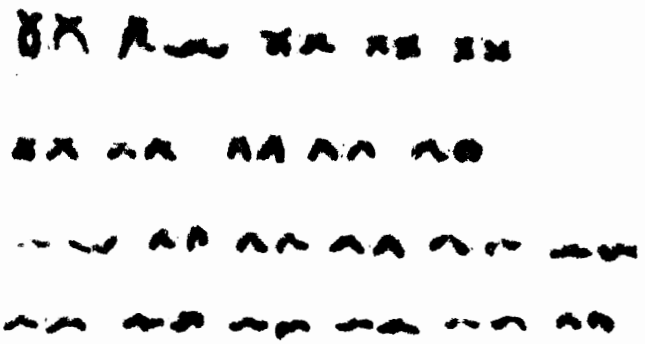

8b

Sehr geehrte KollegInnen,

die Arbeit endet mit Tafel2 (=S. 61). Seite 62 wäre die unbedruckte Rückseite der Tafel. Mit Seite 63 beginnt die nächste Arbeit. Wir haben die Arbeit also vollständig geliefert.

Mit freundlichen Grüßen