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ASSESSING GENETIC STRUCTURE OF PELAGIC FISH POPULATION OF LAKE TANGANYIKA

by
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PREFACE

The Research for the Management of the Fisheries on Lake Tanganyika project (LTR) became fully operational in January 1992. It is executed by the Food and Agriculture Organization of the United Nations (FAO) and funded by the Finnish International Development Agency (FINNIDA).

LTR's objective is the determination of the biological basis for fish production on Lake Tanganyika, in order to permit the formulation of a coherent lake–wide fisheries management policy for the four riparian States (Burundi, Tanzania, Zaïre and Zambia).

Particular attention are given to the reinforcement of the skills and physical facilities of the fisheries research units in all four beneficiary countries as well as to the build-up of effective coordination mechanisms to ensure full collaboration between the Governments concerned.

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TABLE OF CONTENTS

SUMMARY

This paper presents the preliminary results of the study on the genetic structure of pelagic fish populations of Lake Tanganyika.

Specimens of *Limnothrissa miodon* and *Stolothrissa tanganicae,* collected from five different localities of Lake Tanganyika, were studied by RAPD (Random Amplified Polymorphic DNA) method to determine the possible differences between populations.

Specimens of *L. miodon* were tested on four DNA primers. The strains of Malagarasi and Moba share some characters that are missing elsewhere. Similarly, the strains from Chituta and Nsumbu express some characters not found among others.

Specimen of S. *tanganicae* were tested on three primers. On one character, the strains from Rusizi, Malagarasi and Moba differ from strains from Chituta and Nsumbu. On three other characters, the strains from Chituta and Nsumbu resemble the strain from Rusizi.

The DNA-method is reliable to identify even the fry specimens of *L. miodon* and S. *tanganicae.*

Definite conclusions and recommendations for further use of the method can be made only after at least 30 individuals of each population have been analyzed.

1. INTRODUCTION

This report describes the application of the RAPD-DNA method in the study of genetic differentiation of the pelagic fish populations of Lake Tanganyika. Some preliminary notes on genetic diversification between some selected local populations of two species, *Limnothrissa miodon* and *Stolothrissa ta ganicae,* are also given.

Genetic differentiation is an indication of separated life-history strategies and reproduction. The fishes from the same population breed together and thus share the same genetic characters.

The information on the genetic discreteness between the fish populations may eventually be used for fisheries management. If the populations show a significant differentiation in their genetic structure, alternative measures may be adopted for managing the sub-stocks in different parts of the lake.

2. METHODOLOGY

2.1 Sampling

A total of 660 specimens representing 6 species was collected during the summer of 1993 from Lake Tanganyika. Two pelagic clupeids were sampled: *Limnothrissa miodon* (Boulenger, 1906), Figure 1, and *Stolothrissa tanganicae* (Regan, 1917), Figure 2. Also, the nile perch species *Lates stappersii* (Boulenger, 1914), *L. anqustifrons* (Boulenger, 1906), *L. mariae* (Steindachner, 1909) and *L. microlepis* (Boulenger, 1898) were sampled.

FIGURE 1: *Limnothrissa miodon.*

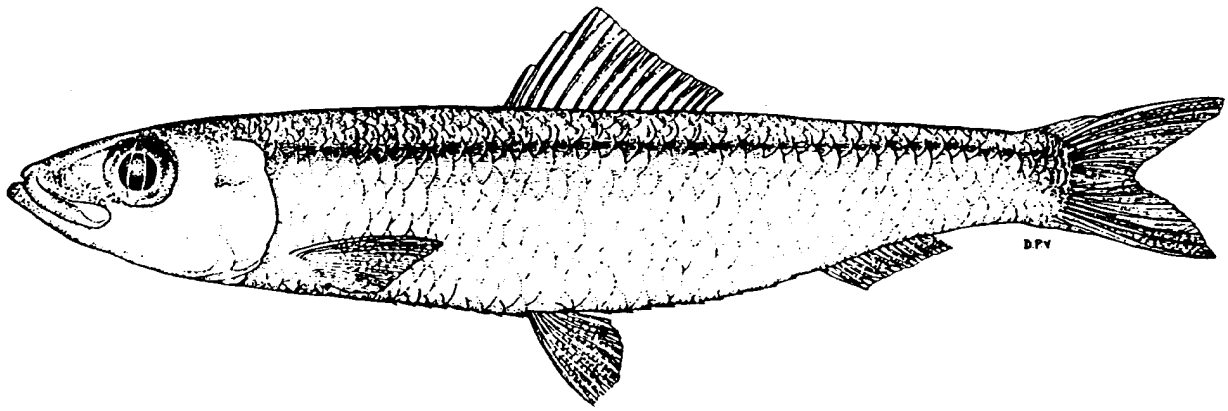
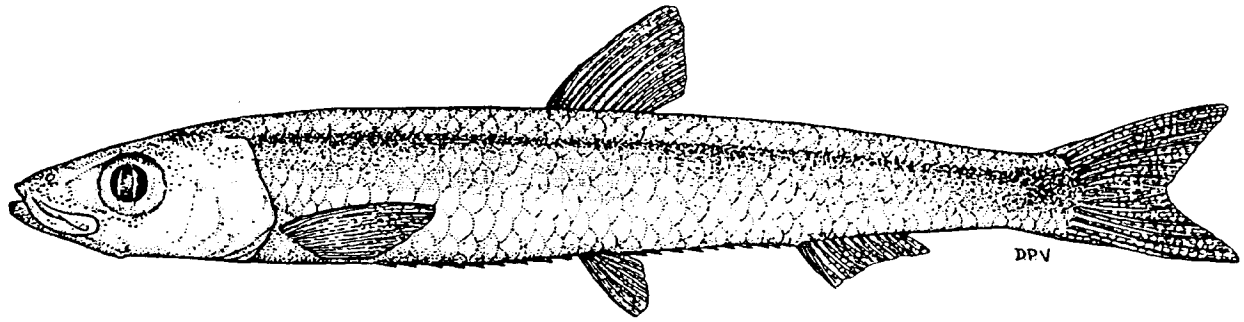


FIGURE 2: *Stolothrissa tanganicae*



Appendix 1 details sampling localities, species and the number of specimens collected. The sampling took place at six localities as shown in Figure 3.

In this preliminary study, only ten *Limnothrissa* and *Stolothrissa* specimens from each locality were analyzed.

Whole individuals *L. miodon* and *S. tanganicae* were stored in equal volumes of pure alcohol. The specimens of *Lates* species were dissected and only muscle tissue was stored in equal volume of pure alcohol. After several days, and after samples became hard, they were removed from alcohol, packed tightly into plastic bags, and sent for analysis. In the laboratory the samples were kept frozen.

2.2 DNA extraction

To study the genetic structure of the pelagic populations, DNA (Deoxyribonucleic acid) must be isolated and purified. This is usually done by using toxic organic solvents, like phenol. In this case, a safe procedure, i.e. the same as normally used for blood samples, was applied. Tissue samples were softened in nuclei lysis buffer (10 mM Tris-HCl, 400 Mm NaCl and 2 Mm Na EDTA, Ph 8.2). They were then digested overnight at 37°C with 0.2 ml 10 % SDS and 0.5 ml of a protease K solution (1 mg/mi protease in 1% SDS and 2 Mm Na EDTA). After digestion was completed, 1 ml of saturated NaCl was added, the tube was shaken vigorously for 15 seconds, and centrifugated at 2500 rpm for 15 minutes. The liquid containing the DNA was transferred to another tube, and two volumes of absolute ethanol, at room temperature, were added. The tubes were inverted several times, until the DNA was precipitating. After DNA had descended to the bottom of the tube, it was removed with a Pasteur pipette to a 1,5 ml Eppendorf tube. The liquid was centrifugated at 13,500 rpm for 3 minutes, the alcohol was removed, and the DNA was then shortly dried. The DNA pellet was dissolved in 1 ml TE buffer (10 mM Tris—HCl, 1 mM Na EDTA, pH

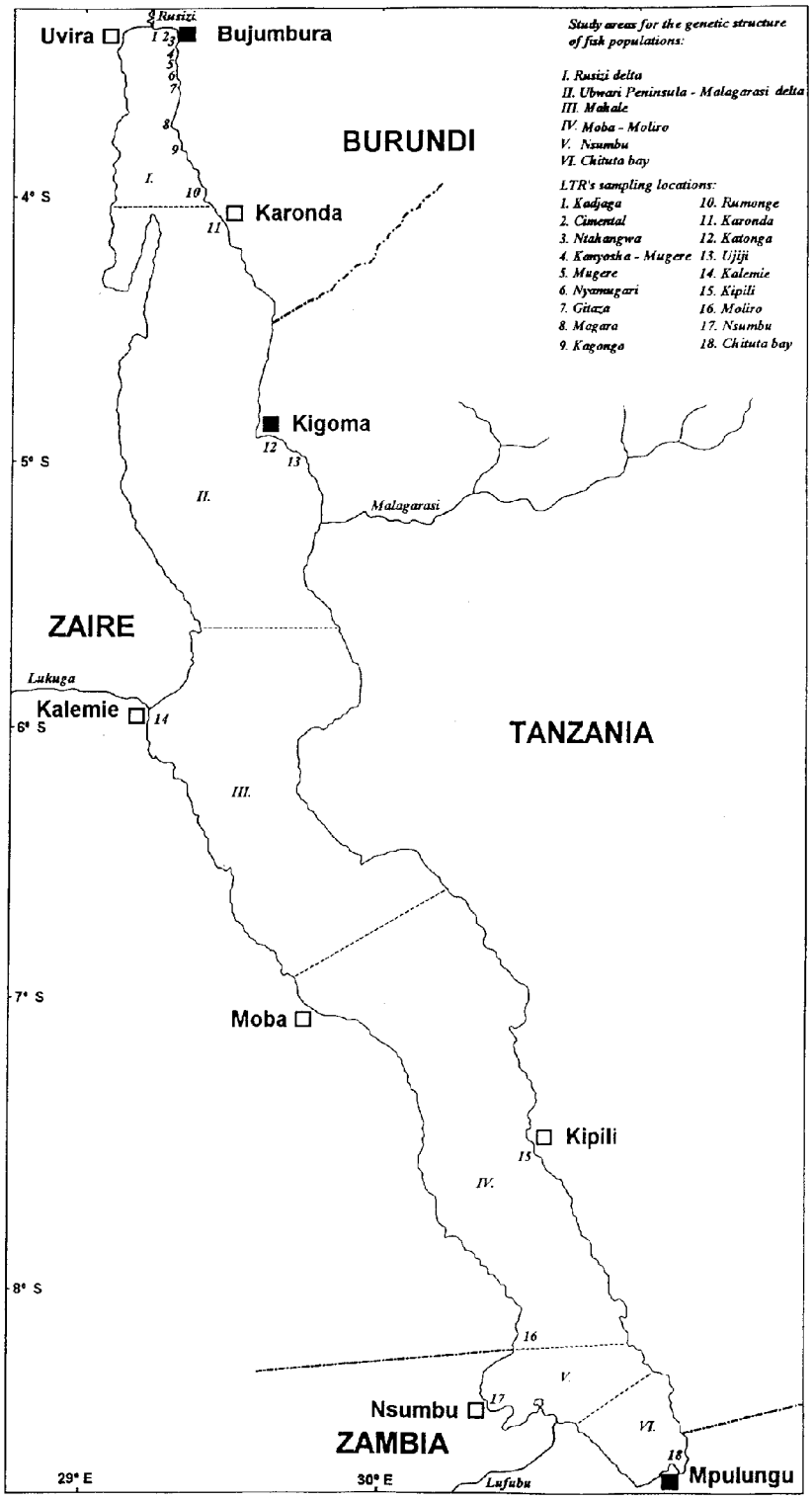


FIGURE 3: Lake Tanganyika:Study areas for the genetic structure of fish populations.

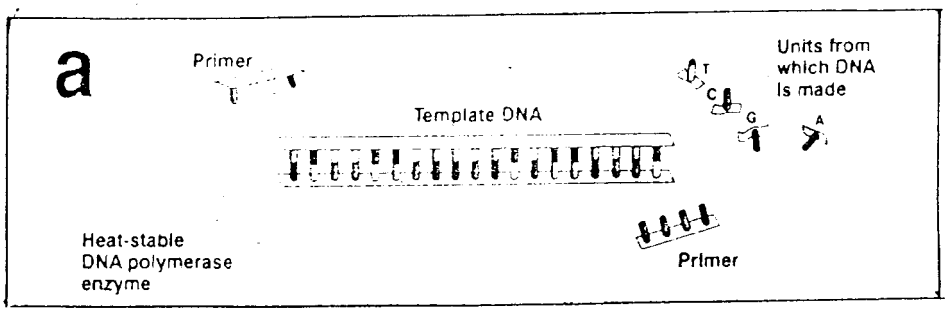
8.0). The amount of DNA was measured with Pharmacia's GeneQuant RNA/DNA Calculator.

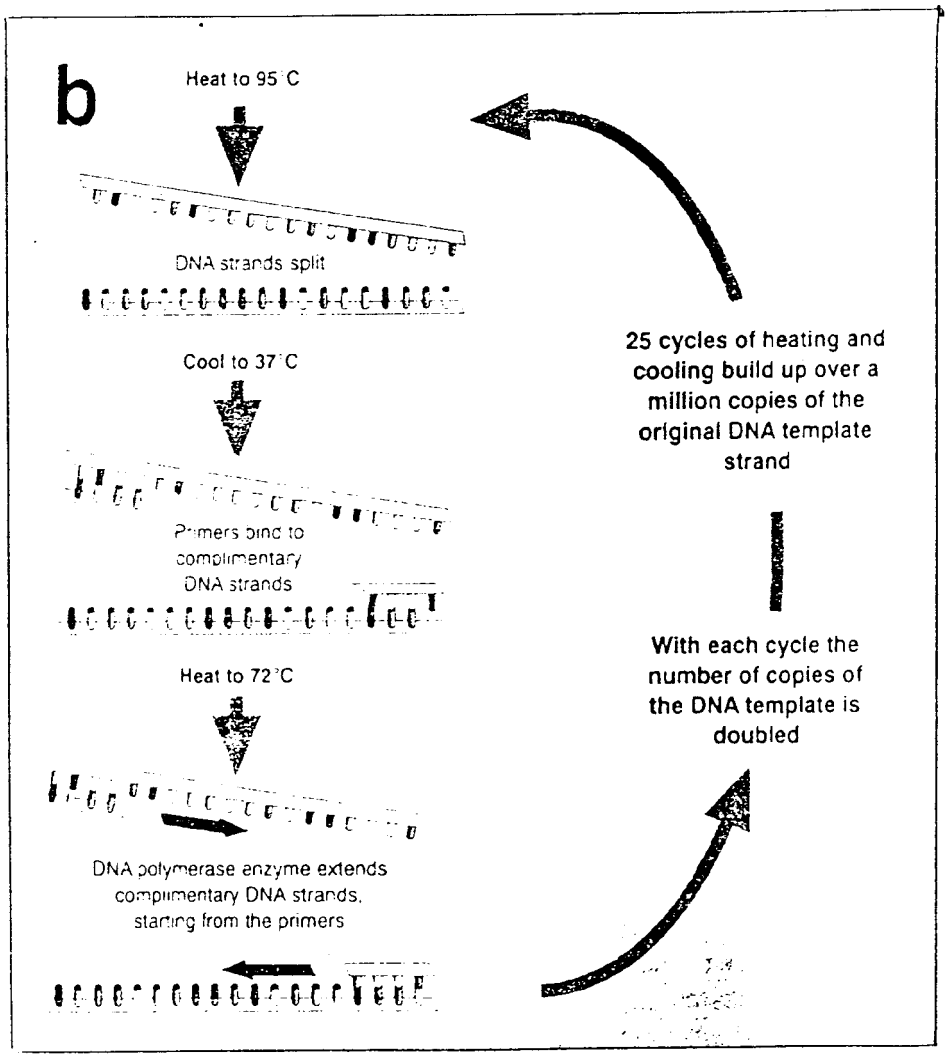
2.3 RAPD technique

The genetic variation between populations was studied by the RAPD (Random Amplified Polymorphic) DNA method (Williams *et al.,* 1990). With this RAPD (rapid) technique, parts of the total DNA are copied. Ten long primer nucleotides copy randomly chosen parts of the total DNA of an individual. The DNA strands are copied on parts where the recipients of the primers are located, less than 3.000 base pairs away from each other. These DNA strands give a random sample of the DNA, representing the total genome of an individual. Thus individuals and populations can be compared, and the total level of genetic identity can be estimated.

Samples of three fish genera, *Limnothrissa, Stolothrissa* and *Lates,* were available in September 1993. Four individuals of *Stolothrissa tan ganicae,* three individuals of *Limnothrissa miodon* and two individuals of *Lates* species were tested with Operon Technologies primers. From the 40 primers which were tested, 20 gave visual bands. Seven primers showed genus specific marker bands. Four of the 40 primers were chosen for comparison of populations. The primers used were OPB 01, OPB 05, OPA 09 and OPA 18 (Operon Technologies). The base codes from 5' to 3' are GTTTCGCTCC, TGCGCCCTTC, GGGTAACGCC and AGGTGACCGT. Strands were polymerized in PCR (polymerise chain reaction), as shown in Figure 4. The temperature profile for PCR was 5 seconds in 94°C, 35 seconds in 36°C and 60 seconds in 72°C. This procedure was repeated 35 times (Yu and Pauls, 1993).

Figure 4: The polymerase chain reaction





The copied parts of DNA were separated and visualised on a 1,4 % agarose gel, where electric current (8 V/cm, 4 A/cm) organize the strands to band according to their size. When these bands are compared with a commercial molecular weight marker (Boehringer-Mannheim MWM VI), the size of copied strands can be counted.

A comparison of the copied parts will reveal small individual differences among the specimens. Some characters of the DNA are shared by the whole flock. Fish that breed together and have the same ancestry, will show the same biochemical pattern. These markers can be used to determine whether all individuals of one species belong to the same population.

Populations and species can be compared by the number and size of strands (Nei, 1987; Hu and Quiros, 1991; Elo and Vuorinen, 1992; Hedrick, 1992; Mohan—Jain *et al.,* 1992).

3. RESULTS AND DISCUSSION

In this preliminary study, *L. miodon* populations shared 30 characters with four different primers. Populations of Malagarasi and Moba shared four bands not found elsewhere. Further, the three most northern populations shared three characters, not found in the southern strains. Three characters that were common in all other populations lacked in Chituta, the southern most population. Chituta and Nsumbu had 5 characters in common, not shared with the others.

These results may be interpreted to reflect some amount of differentiation between the strains. Possibly strains breed locally, with minor mixing from the nearby strains. As samples from the Mahale region in the canter of the Lake were lacking, it was not possible to indicate if this kind of mixing occurs lake-wide. It may be also possible, that the strains of north and south are more or less differentiated from each other.

S. tanganicae populations shared 13 characters with three different primers. Three characters were shared with populations from south, Chituta and Nsumbu, and from up north, Rusizi, but not from the middle populations. Two characters were present only in three northern populations, and one only in the south. The southern most population, Chituta, had five unique characters.

According to these results, the populations of *S. tanganicae* may be mixing more effectively than those of *L. miodon.*

However, these results are based on a very small sample of the populations. From *L. miodon* populations, less than ten individuals have been analyzed; this is far too small a sample to obtain reliable results. Consequently, the observed trends may be strengthened or modified once a thorough analysis is completed.

With RAPID analysis it is easy to discriminate the fry of *L. miodon* and *S. tanganicae.* The banding patterns of these two species are clearly different from each other.

Only the DNA extraction but no variation analyses were done for *Lates* species so far.

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Appendix I: Genetic samples. Populations analyzed in this study are marked with 10 in column Specimens.

GENETIC1.XLS

			GENETIC SAMPLES LTR FIRST SAMPLING 1993					
COUNTRY	DATE	LOCATION	SPECIES	DISTANCE FROM SHORE	DEPTH	# SPECIMENS	GEAR	ZONE
TANZANIA	06.16.93	KIGOMA (Katonga)	Lates stappersii	> 500 m		10 30		Malagarazi
TANZANIA	06.15.93	KIGOMA (Katonga)	Limnothrissa miodon	> 500 m		10 30		Malagarazi
TANZANIA	06.22.93	KIGOMA (Katonga)	Lates mariae	> 500 m		7		Malagarazi
TANZANIA	06.16.93	KIGOMA (Katonga)	Stolothrissa tanganicae	> 500 m		10 30		Malagarazi
TANZANIA	06.24.93	KIGOMA (Katonga)	Lates mariae	> 1000 m		5		Malagarazi
TANZANIA	07.19.93	KIGOMA (Katonga)	Lates mariae	> 1000 m		6		Malagarazi
TANZANIA	07.13.93	KIPILI	Lates stappersii	100-150 m		10 30		Moba
TANZANIA	07.11.93	KIPILI	Stolothrissa tanganicae	50-70 m		10 30		Moba
TANZANIA	07.11.93	KIPILI	Limnothrissa miodon	50-70 m		10 30		Moba
BURUNDI	07.08.93	MAGARA	Luciolates stappersii			10 30	LIFTNET	Ruzizi
BURUNDI	07.06.93	KAGONGO	Stolothrissa tanganicae			30	PURSE SEINE	Ruzizi
BURUNDI	07.08.93	MAGARA	Stolothrissa tanganicae			10 30	LIFTNET	Ruzizi
BURUNDI	07.08.93	MAGARA	Limnothrissa miodon			10 17	LIFTNET	Ruzizi
BURUNDI	07.08.93	MAGARA	Limnothrissa miodon			15	LIFTNET	Ruzizi
BURUNDI	07.06.93	RUMONGE	Limnothrissa miodon			7	BEACH SEINE	Ruzizi
BURUNDI	07.20.93	NTAHANGWA	Lates mariae (1)		25 m	1	GILLNET	Ruzizi
BURUNDI	08.03.93	KANYOSHA-MUGERE	Lates mariae (2)		40 m	1	GILLNET	Ruzizi
BURUNDI	07.13.93	MUGERE	Lates mariae (3)		30 m	2	GILLNET	Ruzizi
BURUNDI	07.06.93	RUMONGE	Lates mariae			20	BEACH SEINE	Ruzizi
BURUNDI	07.06.93	RUMONGE	Lates angustifrons			15	BEACH SEINE	Ruzizi
BURUNDI	07.06.93	RUMONGE	Lates microlepis			3	BEACH SEINE	Ruzizi
ZAMBIA	6-7.93	CHITUTA BAY	Limnothrissa miodon			10 32		Chituta
ZAMBIA	6-7.93	CHITUTA BAY	Stolothrissa tanganicae			10 32		Chituta
ZAMBIA	6-7.93	CHITUTA BAY	Lates mariae			31		Chituta
ZAMBIA	6-7.93	CHITUTA BAY	Lates angustifrons			16		Chituta
ZAMBIA	6-7.93	CHITUTA BAY	Luciolates stappersii			10 30		Chituta
ZAMBIA	6-7.93	NSUMBU	Limnothrissa miodon			10 30		Sumbu
ZAMBIA	6-7.93	NSUMBU	Stolothrissa tanganicae			10 30		Sumbu
ZAMBIA	6-7.93	NSUMBU	Lates mariae			30		Sumbu
ZAMBIA	6-7.93	NSUMBU	Lates angustifrons			30		Sumbu
ZAMBIA	6-7.93	NSUMBU	Lates stappersii			10 30		Sumbu
					Total:	660		
(1) sample taken between rivermouths of Ntahangwa and Mugere								
(2) sample taken in front of rivermouth Mugere (1.1 km south of Bujumbura)								
(3) sample taken in front of rivermouth Ntahangwa (in Bujumbura town)								
ALL SAMPLES TAKEN BY LTR STAFF EXCEPT FOR SAMPLES 1,2 and 3 ABOVE TAKEN BY Dr. L. DEVOS (BELGIUM/CEPGL CRRHA PROJECT)								