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GENETIC VARIATION IN THE POPULATIONS OF PELAGIC CLUPEIDS *Stolothrissa tanganicae* and *Limnothrissa miodon* AND NILE PERCH *(Lates stappersii, L. mariae)* IN LAKE TANGANYIKA

by

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The conclusions and recommendations given in this and other reports in the Research for the Management of the Fisheries on the Lake Tanganyika Project series are those considered appropriate at the time of preparation. They may be modified in the light of further knowledge gained at subsequent stages of the Project. The designations employed and the presentation of material in this publication do not imply the expression of any opinion on the part of FAO or FINNIDA concerning the legal status of any country, territory, city or area, or concerning the determination of its frontiers or boundaries.

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) and the Arab Gulf Program for the United Nations Development Organization (AGFUND).

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, Democratic Republic of Congo, Tanzania, and Zambia).

Particular attention is 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

Abstract

The genetic variation of pelagic fish populations in Lake Tanganyika was studied with material from two endemic nile perch species (*Lates stappersii* and *L. mariae*) and two clupeids (*Stolothrissa tanganicae* and *Limnothrissa miodon*). Genetic differentiation among populations was estimated using the RAPD-DNA method. Three primers amplified 53-61 various DNA fragments from 1012 individuals sampled at six localities. Large variation among RAPD profiles was found, and the substructuring of sampling locations is presented. The differentiation between sampling sites was largest in *Lates stappersii*. Based on RAPD profile similarity, the individuals from different seasons are fairly well separated. Heterogeneity among sampling sites and times was high, but the overall level of genetic differentiation is low, since only a few private alleles were detected. In each species, migration appears to be sufficient to combine all sampled populations to one gene pool.

Introduction

The purpose of this work is to measure the extent of genetic variation within pelagic fish species in Lake Tanganyika and to estimate the level of genetic substructuring in the stocks. Economically important inland fisheries are based on the stocks of these fish in Tanzania, Burundi, Zaïre and Zambia. Of the endemic clupeids, *Stolothrissa tanganicae* is a small and truly pelagic fish, whereas *Limnothrissa miodon* grows slightly larger and lives closer to the shore. Their nile perch predators are the smaller *Lates stappersii* and the large, benthic *L. mariae*. Genetic variation permits local adaptations and provides raw material for evolutionary change. The cichlids in Lake Tanganyika possess high genetic variation (Sturmbauer and Meyer, 1992), and they have diversified enormously (Poll 1956). Endemic species are also known from many other fish families in the lake (Poll 1953).

Protection of any locally adapted fish stocks enhances the overall productivity and sustainable use of fish resources. Suppressed genetic variation may diminish the overall fishery yield (Smith *et al.* 1990), since migration cannot compensate for the loss of genetic diversity and locally adapted genetic resources (Carvalho and Hauser 1994). The stock structure of fish species has been studied with molecular methods like electrophoresis (Casselman *et al.*, 1981, Grant and Utter, 1984, Jørstad *et al.*, 1991), mitochondrial DNA (Bernatchez, 1997, Bentzen *et al.*, 1989, Crosetti *et al.*, 1994, Tringali and Wilson, 1993) and RAPD (Dahle, 1993, Bardacki and Skibinski, 1994, Bielawski and Pumo, 1997, Naish *et al.*, 1995).

Here, structure of the pelagic fish populations in Lake Tanganyika was studied as a part of the FAO:s project "Research for the Management of the Fisheries on Lake Tanganyika", which aims at determining the biological basis for fish production. RAPD (Williams *et al.*, 1990) method was chosen, since it is efficient, economical, uses limited quantities of DNA and works on anonymous genomes (Hadrys *et al.*, 1992). As all these species are streamlined and living in open waters, no presumptions on

the borders of their populations seems justified. This study concentrated on finding the genetic variability to reveal the structure of the stocks.

2. Material and methods

2.1 Clupeid and nile perch samples

A total of 1010 individuals of the four pelagic species were sampled during a dry season (June - August 1993), and during a rainy season (October - December 1993) on Lake Tanganyika (for sites see Fig. 1). A maximum of 30 individuals per species were collected at one site.

Samples were bought fresh from the local fishermen by the Lake Tanganyika Research personnel, and cooled and processed at the fisheries stations. From the *Lates* species a muscle tissue slice of 3x5x1cm surrounding the lateral line was dissected and stored in an equal volume of pure alcohol. Clupeids were stored whole in an equal volume of alcohol.

A total of 262 *Limnothrissa miodon* samples were analysed. Catches were sampled in Magara (Burundi), Kigoma (Tanzania), Kipili (Tanzania), Chituta Bay off Mpulungu (Zambia) and Nsumbu (Zambia) in November 1993 during the dry season. Rainy season samples were gathered from Nyamugari (Burundi), Kigoma (Tanzania), Kalemie (Zaïre), Kipili (Tanzania), Chituta Bay off Mpulungu (Zambia) and Nsumbu (Zambia).

A total of 263 pelagic clupeid *Stolothrissa tanganicae* (Regan 1920) were collected. During the dry season, samples were taken in Magara, Kigoma, Kipili, Nsumbu and Mpulungu. In Kigoma the fishes were caught more than 500 metres offshore, and in Kipili at 50 - 70 m distance from the shore. The samples from Magara are from a liftnet. The fishing method or distance from the shore is not known for the samples from Nsumbu and Mpulungu. During the rainy season October - December samples were collected from Nyamugari near Magara (coded to Bujumbura), Kigoma, Kipili, Kalemie, Mpulungu and Nsumbu.

A total of 270 individuals of the nile perch *Lates stappersii* (Boulenger, 1914) were collected in 1993. During the dry season (June - August) 30 individuals were collected from each of the Magara, Kigoma, Kipili, Nsumbu and Mpulungu sites. During the rainy season (October - December) 30 individuals were sampled from Kipili, Mpulungu and Nsumbu, 10 from Magara and 20 from Gitaza, a site about 10 km north from Magara.

Lates mariae was sampled during the dry season in Rumonge, Mugere, Ntahangwa (Burundi, coded to Bujumbura in the graphs),

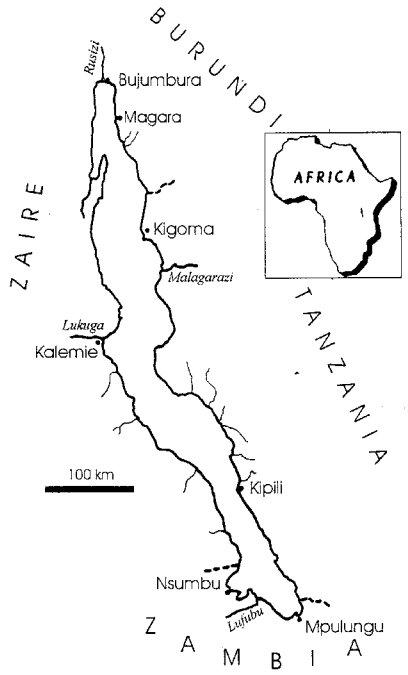

Figure 1. Map sshowing the sampling sites in Lake Tanganyika. Gitaza, Rumonge, Mugere, Ntahangwa, Karonda, Kadjaga, Cimenta and Nyamugari are in the vicinity of Magara in Burundi.

in Kigoma (Tanzania), Mpulungu and in Nsumbu (Zambia). During the rainy season, samples were caught from Mugere, Karonda, Kadjaga, Cimental, Nyamugari (Burundi, coded to Bujumbura in the graphs), Kigoma (Tanzania), Moliro (southern Zaïre, coded to Kipili) and from Mpulungu (Zambia). A total of 217 individuals were analysed.

2.2 DNA extraction

The DNA for each population was extracted on different days to prevent the possibility of contamination. DNA was extracted from a few mm^3 piece of muscle tissue cut out from the inner part of the preserved slice or individual. The use of toxic organic solvents was avoided by applying the method of Miller *et al.* (1988). The amount of DNA extracted varied between 15-150 micrograms per millilitre. The quality of the DNA was examined by the ratio of absorptions A260 / A280. All samples were diluted to a concentration of 5 nanograms of DNA / microliter for PCR.

2.3 RAPD protocol

For each species, sixty primers were tested with two individuals from different localities. A single, 10 base-pair long primer amplified sequencies, where the primer target sites on opposite DNA strands were less than 3,000 bp away from each other (Park and Moran, 1994). The resultant presence or absence of amplification products gives a random sample of the total DNA of an individual, and the level of genetic similarity of individuals and populations can be estimated. The primers for

population analysis were selected by their clear and repeatable pattern.

DNA strands were polymerased by PCR in a total volume of 25 µlitres with 10 ng of template DNA, 4 pmol of a single primer, 1 unit of Tbr DNA polymerase (*Thermus brockianus*, Finnzymes, Finland) and 100 mM each of the four dNTPs (deoxynucleotide triphosphates, Promega or Finnzymes) in the buffer (10 mM Tris-HCl, pH 8.8 at 25°C, 1.5 mM $MgCl_2$, 50 mK KCl, 0.1% Triton X-100). The temperature profile for the PCR was 5 seconds 94°C, 35 s. 36°C and 60 s. 72°C, repeated 35 times (Yu and Pauls, 1993). A negative control without template-DNA was present in all amplifications. One gel consisted 54 individuals from at least two populations. The copied parts of DNA were separated and visualised with ethidium bromide on 1.4% agarose gel with a molecular weight marker (Boehringer-Mannheim MWM VI). An electric current (8 V/cm, 4 A/cm) organized DNA strands into bands according to their size. All utensils and liquids were autoclaved prior to the use.

2.4 Analysis of RAPD data

All fragments on gels were coded as absent (0) or present (1). Thus a binary matrix was created, in which each row contains the genetic information for each individual. Due to the lack of normality in this binary data, the independence of bands was estimated using the Spearmans rank correlation coefficient (Sokal and Rohlf, 1981).

The heterogenity among samples in one species was estimated by counting first pairwise, the binary squared Euclidean distances for all individuals and then counting the coefficient of variation CV among them. The effect of chance as a cause for heterogeneity was estimated from the original matrix by bootstrapping with 1000 randomizations using the program REAP (Roff and Bentzen, 1989, McElroy *et al*. 1991).

In order to determine the effect of band number to the estimates of genetic relationships, a bootstrap analysis was performed using data from *Stolothrissa tanganicae*. First, 200 new matrices were constructed by using randomly four to sixty bands. Then pairwise relationships for all individuals in each new matrix were counted using fraction of matches index M (Black 1994b). Finally, the mean coefficient of variation of similarities was plotted against the number of bands used (Thormann *et al.* 1994).

Observed bands were interpreted to allele frequency data with the program RAPDBIOS (Black 1993) after following assumptions: 1) comigrating bands arise from identical alleles, 2) absence of a band is caused by an identical ancestral mutation, 3) bands are independently segregting loci, 4) presence of a band represents a dominant homo- or heterozygote locus and 5) that the genotypes are in Hardy-Weinberg equilibrium. The allele frequencies were used in the BIOSYS (Swofford, 1989) program to estimate levels of variation, and relationships between populations as Nei’s genetic distances. With the same

assumptions the genetic differentiation and migration levels between geographic areas and sampling sites were estimated using the program RAPDFST (Black, 1994a). F_{ST} is counted as the ratio of the observed variance in the frequency of an allele among subpopulations relative to its maximum variance in the total population, estimated by the formula F_{ST} = variance (p) / (average p (1- average p)), where p is the frequency of an allele at a RAPD locus and average p is the weighted average frequency among all subpopulations.

Effective migration rate Nm = (1 - F_{ST})/ 4 F_{st}.

The great number of variants achieved with RAPD allows the use of statistical methods. Principal component analysis (Noru_is, 1995) was used to summarize the variables to a smaller number of underlying factors and nonparametric Kolmogorov-Smirnov 2-sample test was used to test the similarity of band frequencies in two populations. This test was used instead of x^2 due to the lack of observations in some classes of binary variables (no bands observed). The test is based on maximum absolute difference between the observed cumulative distribution functions for both samples, and it is sensitive to any differences in the two distributions: shape, location etc. Table-wide sequential Bonferroni test was used to estimate the accuracy of significance levels (Rice, 1989).

Differences between single individuals were calculated with the fraction of matches index M, recommended for intraspecific comparisons (Apostol *et al.* 1993, Black 1994b), when M = N_{AB} / N_T, where N_{AB} is the number of shared presence and absence of bands in individuals A and B, and N_T is the total number of bands detected in the species (Black 1994b). A hierarchical clustering of distances was drawn in the form of a UPGMA dendrogram using Neighbor program in PHYLIP package (Felsenstein, 1993)

To finally estimate the relevance of the detected variation, effective migration rate was counted using Slatkin's index p (Slatkin, 1985). Slatkin's index p is based on the frequencies of alleles found in only one deme, i.e. on private alleles, without other assumptions.

3. Results

The RAPD technique revealed rich variation in two pelagic clupeids and two nile perches apecies from Lake Tanganyika. When the DNAs of two individuals from different parts of the lake were amplified with three primers from Operon Technologies primer sets OPA, OPB and OPC, about 60% of the primers gave visual bands with all species. Three primers producing a clear and reliably reproducing banding pattern were chosen for population analysis. The number of bands observed with three primers ranged from 61 in *L. miodon*, 60 in *S. tanganicae*, 58 in *L. stappersii* to 53 in *L. mariae.* Detected fragments and the primers used are shown in appendix 1. Primary data for each species is given in appendices 2 - 5.

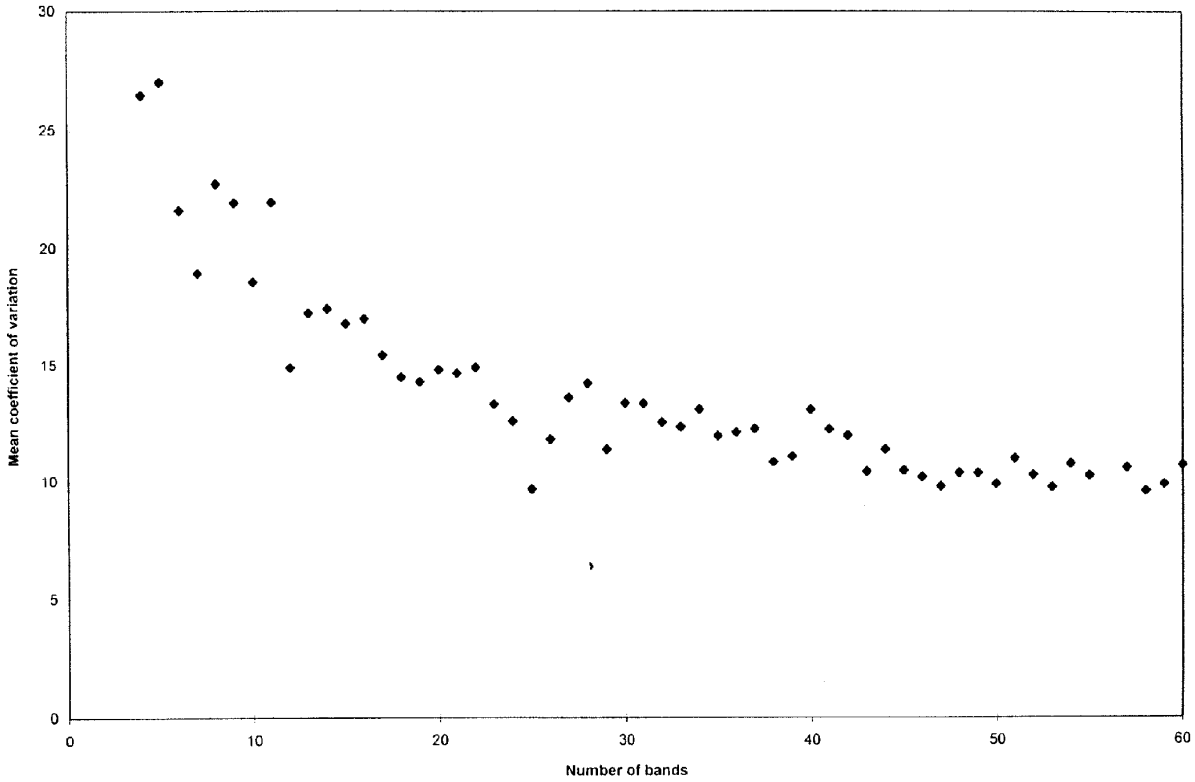

Figure 2. The effect of band number to the estimates of genetic relationships in *Stolothrissa tanganicae* data. 200 new matrices were created by bootstrapping and the mean cv of similarities was plotted against the number of bands used.

The presence of a band was to a large extent independent of other bands, since the Spearman correlations among the bands were mostly below 0,5. The following bands possessed correlations higher than 0.7: 4 correlations among bands of *L. miodon* (X17, X18, X19, X28, X39), 3 in *S. tanganicae* (X15, X17, X19, X20, X55, X60), 25 in *L. stappersii* (X12, X13, X15, X16, X18, X23, X25, X34, X40, X45, X46) and 13 in *L. mariae* (X4, X6, X8, X9, X12, X14, X16, X20, X21, X26, X30, X32, X36, X37). Exclusion of these bands did not affect the pattern of similarity.

The heterogeneity among species, when measured as a coefficient of variation among pairwise similarity values between individuals, was high in Nile perches; 0.60 in *L. stappersii* and 0,56 in *L. mariae*, and lower in clupeids; 0.28 in *S. tanganicae* and 0.29 in *L. miodon.*

The variation among species was not a result of chance alone, since in Monte Carlo simulations with 1000 randomizations of the original data of sampling, the probability of exceeding the original $?^2$ by chance was less than 0.000 in all studied species.

The observed number of bands seems sufficient for the estimation of genetic similarity. In the bootstrap test on the similarity values, the coefficient of variation decreases when more bands are used, and levels off when more than 50 bands are used (Fig. 2).

Table 2. Genetic variation as the percentage of loci polymorphic

Location	*L. miodon*	*S. tangan.*	*L.stapp.*	*L. mariae*
Bujumbura	67.2	75.0	56.9	61.5
Kigoma	52.5	60.0	56.9	55.8
Kalemie	41.0	66.7		
Kipili	77.0	80.0	39.7	28.8
Mpulungu	62.3	65.0	53.4	71.2
Nsumbu	63.9	70.0	46.6	55.8

When samples taken on the same area are united to one local sample, and RAPD fragments are interpreted to allele frequencies after several assumptions, the relationships of populations can be presented in an UPGMA dendrogram utilizing Nei's distances (Fig. 3). Dendrograms illustrate the populations of *L. miodon* split to two groups, and *S. tanganicae* samples from dry east coast on their own branch. The dendrogram of *L. stappersii* strikingly separates the Kigoma population from the others. The *L. mariae* dendrogram separates samples from dry Mpulungu and rainy Kipili from others, and shows the remaining samples in groups of dry and rainy season. The level of genetic variation is high, as very many loci were polymorphic in all species: 41-77% in *L. miodon*, 60-80% in *S. tanganicae*, 39-56% in *L. stappersii* and 28-71% in *L. mariae* (Table 2). Due to the high variation observed among samples, the justification for uniting temporally different samples was not self-evident.

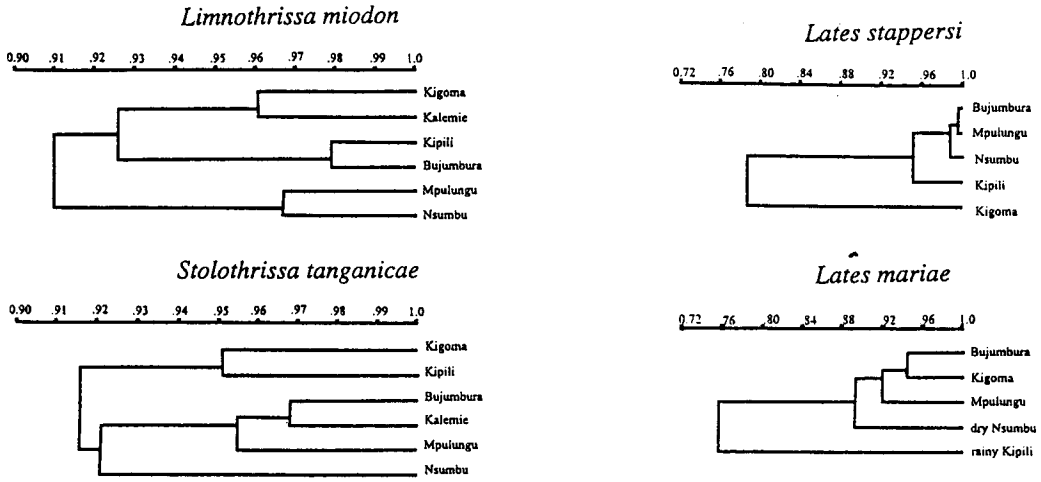

Figure 3. Substructuring of geographic areas in UPGMA dendrograms based on Nei's identity.

After separating temporal samples, the dendrogram patterns changed slightly, since dry and rainy season samples from the same locality were not the most similar (Fig. 4). Treating each sampling occassion as a separate unit lowers the polymorphism of loci, but some are still quite high: *L. miodon* 29-60%, *S. tanganicae* 38-66%, *L. stappersii* 32-56% and *L. mariae* 28-56% (Table 3). In *L. miodon* the dry season samples from the north

end (Bujumbura) cluster with the southern end (Nsumbu and Mpulungu). Rainy season samples from the north-central waters form their own group. In *S. tanganicae* three groups branch off from the rest: samples from the dry east coast (Kigoma and Kipili), rainy south west (Nsumbu) and Malagarazi delta (Kigoma). Dry season samples from the north and south ends are similar. In the *L. stappersii* dendrogram the dry season sample from Kigoma remains distant from the others, and the dry season samples from north and south ends are similar. In the *L. mariae* dendrogram rainy Kipili and dry Mpulungu differ from the others. Also in *L. mariae* the dry season samples from the north end are similar to samples from dry Nsumbu (south west).

Table 3. Genetic variation at all sampling sites

Sampling sites	*L. miodon*	*S. tangan.*	*L.stapp.*	*L. mariae*
dry Bujumbura	45.9	63.3	56.9	28.8
rainy Bujumbura	55.7	65.0	53.4	50.0
dry Kigoma	29.5	38.3	56.9	38.5
rainy Kigoma	27.9	46.7		36.5
rainy Kalemie	41.0	66.7		
dry Kipili	52.5	65.0	32.8	
rainy Kipili	60.7	58.3	39.7	28.8
dry Mpulungu	32.8	50.0	43.1	48.1
rainy Mpulungu	47.5	50.0	48.3	30.8
dry Nsumbu	54.1	61.7	39.7	55.8
rainy Nsumbu	42.6	50.0	43.1	

Migration levels estimated with RAPDFST (Black, 1994a) between different geographic areas were not very high, but sufficient to suppress genetic differentiation: for *L. miodon* Nm is 1.6, for *S. tanganicae* 1.7 and for *L. stappersii* 1.1. For *L. mariae* the migration level is so low, 0.8, that differentiation is possible. If samplings from different seasons are treated separately, migration rates decrease: (Nm for *L. miodon* 0.8, *S. tanganicae* 0.6, *L. stappersii* 0.6 and *L. mariae* 0.4) thus increasing the change of genetic differentiation.

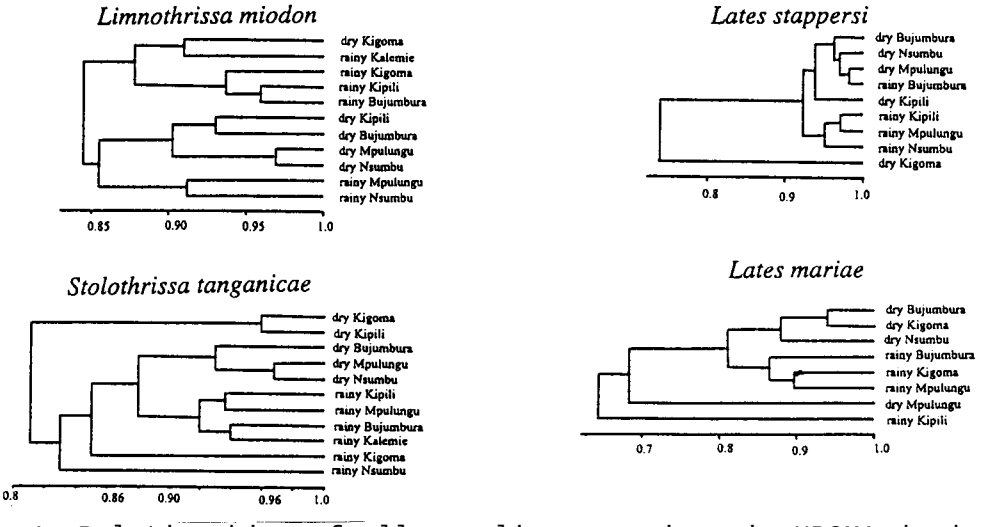

Figure 4. Relationships of all sampling occasions in UPGMA dendrograms based on Nei's identity.

The great number of RAPD bands allowd the use of statistical methods. In a principal component analysis for *L. miodon,* samples from the rainy south end are separate from the rest (eigenvalues 6.3; 5.3; 4.3)(Fig. 5). This analysis was not very well suited for clupeids, since eigenvalues decreased slowly also in *S. tanganicae* (6.4; 5.2; 5.0). In a three dimensional figure (Appendix 6) samples from rainy Nsumbu are separated from the others. The second group differentiating in PCA is formed from individuals from Bujumbura, Kigoma and Kipili caught during the dry season. In the principal component analysis of *L. stappersii* (Fig. 6) eigenvalues for the two first factors were high (13.56 and 6.30) and resulted in a clear separation between 25 samples from Kigoma and all the others. PCA of *L. mariae* showed clearly the separation of most populations with high eigenvalues (9.4 and 7.3).

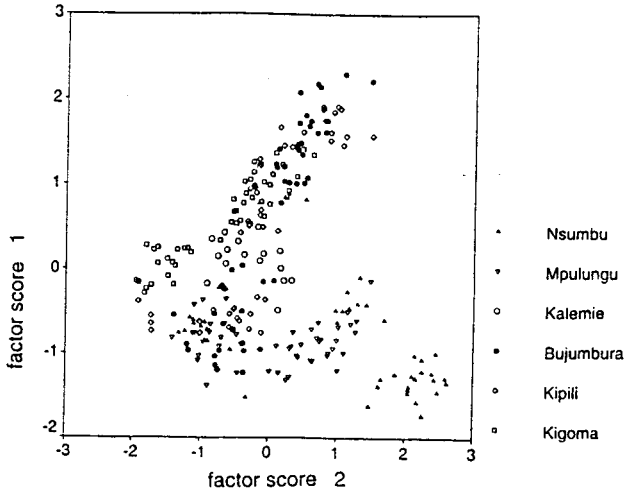

Figure 5. Principal component analysis on *Limnothrissa miodon*

The individuals were clustered also based on the similarity of their banding patterns. UPGMA dendrograms based on percentile similarity values M between single individuals are shown in Figs. 16 -19. In several cases samples from the same locality split into several clusters. Levels of heterozygosity, genetic

distance and migration are smaller for this alternative grouping, but this is self evident, since groups are based on to similarities. In the *L. stappersii* samples this alternative grouping confirmed the difference between the main population and the Kigoma samples. Five individuals sampled in Kigoma during the dry season differ from other local samples. This difference was statistically significant in 9,3 % of bands after the table-wide sequential Bonferroni test. These five individuals in Kigoma appear to be members of the main population, as sequential Bonferroni test proved their band frequencies to be similar. On the contrary the band frequencies of the remaining 25 individuals from Kigoma are different from the main population in the two-way Kolmogorov-Smirnov test. Correlating bands were excluded from these tests.

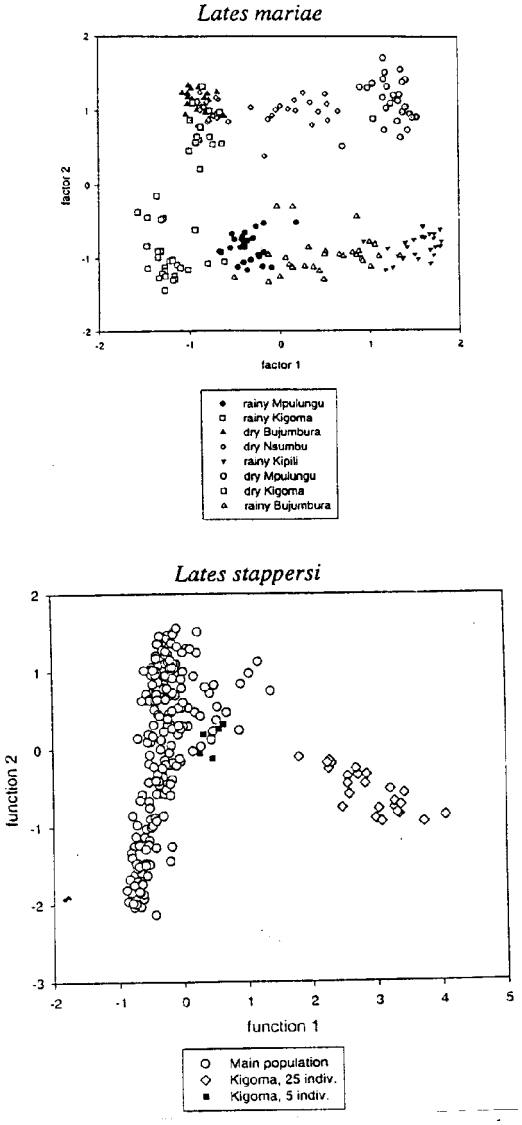

Figure 6. Principal component analysis on Nile perch

The private alleles method gives very similar estimates of gene flow as the F_{ST} (Slatkin & Barton, 1989), but without assumptions required by Black (1994a). According to Slatkin (1985) the low number of private alleles found in all studied species reflects a high migration rate (lowest Nm = 92 for *L. stappersii*), which prevents differentiation by drift in studied

demes. According to this, the relevance of observed variation is small. Only 1 private allele was detected in *L. miodon* and *L. mariae*, 3 in *L. stappersii* and 0 in *S. tanganicae*. Groups based on the similarity of single individuals (Appendix 7.) result in same numbers of private alleles. Based on Slatkin's index p, the level of effective migration (Nm) in *L. stappersii* is 92, and more in other species.

4. Discussion

4.1 Biology of species

Endemic clupeids of Lake Tanganyika share many of the features of their marine relatives: thay are small, short-lived, fecund planktivores, who are attracted by light and having fluctuations in abundance (Coulter, 1991). Annual vertical mixing of lake water nourishes the plankton blooms and gives rise to the cohort of *S. tanganicae* (Coulter, 1991). Eggs and larvae of *Stolothrissa* are pelagic, and from early on follow the adults in daily vertical migrations from surface to 100-200 depth (Coulter, 1991). *Limnothrissa* is more common in shores with a shelf area, like in Zambian waters. It hatches near the shores, and moves to pelagic zone in one years age. There it feeds also on *Stolothrissa*, and takes part in vertical movements (Coulter, 1991).

Lates stappersii is a mackerel-shaped endemic predator of the pelagic zone of Lake Tanganyika. It is the smallest (48 cm) of the endemic Nile perches in Lake Tanganyika (Coulter 1991). It preys on *Stolothrissa* and seems to be truly pelagic, as the eggs contain an oil droplet (Coulter 1991). *L. stappersii* has dence schools and seasonal high catches (Coulter, 1991). It feeds on the sardine-like endemic clupeids when they are abundant, and switches to shrimps when they are not (Coulter, 1991). Since the decline of larger endemic *Lates* species (Coulter, 1991) its importance in fisheries has increased, and the genetic structure of the stock has become an important issue for sustainable use of the stock. The big eyed *L. mariae* is the top predator of the benthic zone. It has a juvenile inshore phase, and grows very large (90cm). It lives very deep, near the anoxic water layer, up to 215 m, but also follows at night the clupeids to the surface. The catches of large endemic Lates-species, including *L. mariae*, have drastically declined since the invasion of modern fishery (Coulter, 1991).

4.2 Reliability of results

Difficulty in interpreting the results originates from the method, since although RAPD bands are genetically defined, they do not represent any known part of the genome, but reveal information on the divergence of the genome as a whole (Stammers *et al.* 1995). RAPDs are unlikely to be strongly linked to coding DNA (Isabel *et al.* 1995) or loci that are under selection pressure (Stammers *et al.* 1995). They are difficult to compare with methods, where the genetics of a known functional system represents the whole genome. The detected high variation among pelagic species of Lake Tanganyika is typical for RAPD as it

reveals much higher variation then electrophoresis, RFLP or mtDNA (Liu and Furnier 1993, Halldén *et al.* 1994).

The problem of achieving reproducing results is solved with rigorous attention to detail (Heun and Helentjaris 1991, Hadrys *et al.* 1992, Coffroth and Mulawka 1995, Skroch and Nienhuis 1995) and by keeping the critical steps constant: enzyme source and concentration, thermocycler, Mg-ion concentration and amount of DNA template (Carlson *et al.* 1991).

RAPD fragments are phenotypic markers inherited in a Mendelian fashion (Williams *et al.* 1990). When a fragment does not appear, the reason may be a point mutation on the primer annealing site, change in the distance between the primer annealing sites or absence of the amplified fragment. Fragments appear to be homologous sequences within species (Thormann *et al.* 1994) allowing the comparison of populations.

The primers were selected purely by the amount of variability they produced. As no strong presumptions were made on the population borders for these high mobile fish, it is possible, that primers with resolution between populations (Hadrys *et al.* 1992, Bardacki and Skibinski 1994, Haymer and McInnis 1994) were neglected. Selecting the primers by their variability lowers Nei's identity values. Primers amplified many rare bands, which still decreased the values of Nei's identity (Wright, 1978). Another reason is the dominant nature of RAPD markers, which leads to underestimates of recessive alleles. Each RAPD fragment corresponds to one locus with two alleles: dominant presence or recessive absence of the fragment (Chalmers *et al.*, 1992, Hadrys *et al.*, 1992, Thormann *et al.*, 1994). Monomorphic recessives cannot be detected at all, if no dominants are present in the sample (Liu and Furnier 1993). The dominant nature (95% of bands by Williams *et al.* 1990) of fragments was impossible to verify, since no haploid tissue was available for comparison. With verification from haploid tissue, heterozygosities and population fixation indices based on RAPD, are in complete agreement with isoenzyme results (Isabel *et al.* 1995). Strong assumptions in the interpretation of the RAPD band profiles to allele frequency data include that genotypes are in Hardy-Weinberg equilibrium in each population. These assumptions make the values of Nei's identity and F_{ST} (Black, 1994a) as being only suggestive and very sensitive to the probable deviations. The following conclusions are based on presumptions that the detected RAPD variation implies a realistic picture of population differentiation, and the sampling has given an accurate view of the stock.

4.3 Genetic diversity in pelagic populations of Lake Tanganyika

In this study RAPD revealed no significant differentiation among populations of pelagic fish in Lake Tanganyika, although it has been very efficient in the identification of species and subspecies of tilapia, *Drosophila*, nematode, molluscs, poplars and mosquitos (Ballinger-Crabtree *et al.* 1992, Caswell-Chen *et al.* 1992, Casiglione *et al.* 1993, Crossland *et al.* 1993, Bardakci and Skibinski 1994, Zande and Bijlsma 1995). The

geological history of Lake Tanganyika shows, that several opportunities for allopatry and genetic divergence of fish have taken place (Tiercelin and Mondeguer, 1991). The first low synrift during Miocene (24 MY ago) consisted of three large basins: one in the north at the mouth of Rusizi, one central basin around the mouth of Malagarasi, and the southern basin. During Pliocene (4.6 MY ago) the central and southern basins were more strongly divided by the rising Kalemie block. The southern basin was divided into two main parts in the Middle Pleistocene, 200 000 years ago (Tiercelin and Mondeguer, 1991).

When individuals were grouped by the similarity of their RAPD profile, the groups formed came from many sampling sites. The pattern of cluster analysis joining samples from different areas and seasons is familiar also in northern anchovy, where the genetic structure is nonrandom, but not identifiable (Hedgecock *et al.*, 1994). Also this species is mobile, and sampled in the pelagic, not in the breeding area. The value of the similarity index within clusters of clupeids (Appendix 7) is very close to those separating plant and animal strains and cultivars (Apostol *et al.* 1993, Dweikat *et al.* 1993, Puterka *et al.* 1993, Rus-Kortekaas *et al.* 1994, Dawson *et al.* 1995), as well as to the theoretical value of 85% for siblings (Apostol *et al.* 1993). Thus it is possible, that these alternative groups represent relatives. Based on high level of differences in band frequencies among groups, the members of these groups can be identified according to the presence or absence of fragments.

If these differencies have a long history, the populations should be genetically differentiated, and possess some private alleles. As this was not observed, it seems most probable, that shoals are temporal structures. The genetic combinations in each new generation are unpredictable, resulting from stochastic sampling of parents. The uneven breeding success of pelagic fish with a high number of eggs, combined with haphazard recruitment of larvae can quickly alter the gene frequencies of a population (Johnson and Black, 1982, Hedgecock *et al.*, 1994). This phenomenon may be responsible also for results from mtDNA studies on *Limnothrissa miodon*, where the highest differences were detected from samples within a very close geographical vicinity (Hauser et al. 1998). There is some evidence for individuals beeing related within schools in anchovy *Engraulis encrasicolus* based on multilocus DNA fingerprinting (Carvalho *et al.*, 1994), but this phenomenon was not detected in minnow *Phoxinus phoxinus* (Naish, 1993).

According to Slatkin’s index, migration between strains is sufficient to prevent genetic separation. Roughly speaking, one individual, exchanged every other generation between local populations is enough to eliminate the effect of alienating genetic drift (Slatkin, 1985). Perhaps some conditions on the lake favour migration, since a clear coherence in dry season samples from opposite ends of the lake was detected among all species studied. This seems to imply lively migration (more than one individual per generation) between areas as far as 600 - 700 km apart. If this finding is confirmed by other methods, it proves of effective gene exchange over large areas. An

interesting question is, at what time of the life cycle migration takes place.

According to the RAPD variation detected in *L. miodon*, populations of this less pelagic clupeid practise enough gene exchange to avoid local differentiation. Only samples caught during the rainy season from the southwest end differ from the main stock. Differences in band frequencies are statistically significant, but the absence of private alleles denies genetic differentiation. In *L. miodon* breeding activity has a locally different timing: In northern end of the lake August - October, December, January and March, in Kigoma September and Mpulungu January - June (Aro and Mannini, 1995). In the study of Hauser *et al*. 1998 the genetic structure of *L. miodon* was studied utilising morphometrics, allozyme electrophoresis and RFLP on mtDNA. Their conclusion was, that *L. miodon* has nonrandom associations of individuals in temporally stable schools, but no genetic differentiation in larger geographic scale. And as now, the existence of self-recruiting populations cannot be discounted. School consisted of fish of similar size and shape, but the high variation in maternally inherited mitochondrial DNA denied their close relatedness. Differences among schools may reflect their origin on different nursing areas.

The observed variation in *S. tanganicae* may imply a slight differentiation of two strains from the main stock: one caught in Nsumbu and the other from Kigoma, Kipili and Bujumbura during the rainy season(Appendix 6.). The clupeids seem to breed all year round, since ripe individuals have been caught during every month (Aro & Mannini 1995). The actual breeding sites of *S. tanganicae* are unknown, but individuals near maturity are caught at different times in different parts of the lake (Aro and Mannini, 1995). The number of ripe females is high twice a year in the north and south ends, so it may be possible that generations from the early and late rainy season are slightly different. The differentiation of temporal samples of clupeids may reflect the fact, that small clupeids born in the rainy season may be too small to be caught during the dry season. All studied strains are genetically combined by migration.

Many methods differentiated *Lates stappersii* stock of Kigoma from the main population: bandsharing values, principal component analysis, dendrogram and differencies in band frequencies. The overall level of migration among *L. stappersii* sampling sites is high, but when this 25 individual sample from Kigoma is compared with combined samples of the main stock, the level of migration is very low (Nm = 0.43).

The restricted gene flow between Kigoma and other populations does not affect Slatkin’s index when all sampling sites are compared, due to the high variability among the main stock and to the lack of several alleles (23%) from the Kigoma sample. But combining variable samples in to a ’main stock’ is probably not justified, as a real isolation of Kigoma population should have produced more than one private allele. Thus the stock from Kigoma is not isolated from the main stock, but with the observed high level of differencies in band frequencies, the members of Kigoma population can be identified with a high

probability according to the presence or absence of fragments. Among other pelagic fish genetic separation of oceanic and local coastal stocks has been observed in herring (Jørstad *et al.* 1991). The breeding biology may reflect this differentiation, since the time for breeding is different in the central and southern areas (Aro and Mannini, 1995). Highest number of spawners was observed in Mpulungu in March and in Kigoma in August (Aro and Mannini, 1995). Maturity is reached in the north at a length of 290 mm (Roest, 1985), and in the south at 210 mm (Ellis, 1978). Fishing pressure is highest in the northern part of the lake, and the catches are very much influenced by the recruiting cohorts (Aro and Mannini, 1995).

Based on RAPD variation *L. mariae* seems to have fairly diversified populations, especially in the south, at Mpulungu and in Kipili. The stock seems to consist of dry and rainy season lineages, but the number of private alleles in each population was very low, and thus the migration between populations is sufficient to prevent genetic differentiation.

The stocks of studied pelagic species of Lake Tanganyika are not genetically random, but the amount of migration is enough to prevent genetic differentiation. Similar results with some genetic differentiation but no isolation has been observed in several marine pelagic fish: jackass morwong *Cheilodactylus macropterus* (Richardson, 1982), South African anchovy *Engraulis capensis* (Grant, 1985), skipjack tuna *Katsuwonus pelamis* (Ward, 1995), and northern anchovy *Engraulis mordax* (Hedgecock *et al.* 1994). However, migration which is sufficient to prevent genetic differentiation, is not necessery enough to assure sustainable fisheries.

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Appendix 1. Primers used and RAPD fragments detected

Primers used and fragments detected

Limnothrissa miodon
OPB-01 5' -GTTTCGCTCC-3' : 2200, 1800, 1700, 1650, 1400, 1300, 1200, 1150, 1070, 1000, 940, 880, 830, 800, 780, 640, 530, 490, 460, 430.
OPA-09 5' -GGGTAACGCC-3' : 1600, 1550, 1400, 1350, 1300, 1170, 1100, 1050, 1000, 940, 880, 860, 780, 760, 680, 640, 580, 520, 490, 430.
OPA-18 5' -AGGTGACCGT-3' : 1750, 1550, 1400, 1350, 1270, 1200, 1100, 1050, 980, 940, 920, 900, 880, 840, 800, 740, 700, 660, 620, 580, 560, 500.

Stolothrissa tanganicae
OPA-18 5' -AGGTGACCGT-3' : 2100, 1750, 1400, 1350, 1200, 1150, 1060, 940, 860, 840, 780, 670, 640, 600, 560, 490, 440, 420, 340, 300.
OPC-02 5' -GTGAGGCGTC-3' : 1550, 1450, 1250, 1150, 1100, 1020, 920, 900, 850, 820, 760, 720, 680, 650, 600, 580, 520, 480, 460, 350.
OPC-08 5' -TGGACCGGTG-3' : 1750, 1400, 1350, 1250, 1150, 1100, 1050, 960, 900, 840, 820, 800, 750, 720, 660, 640, 610, 520, 460, 370.

Lates stappersi
OPC-08 5' -TGGACCGGTG-3' : 1030, 900, 850, 760, 700, 640, 600, 520, 480, 450, 400, 350, 340, 260.
OPC-13 5' -AAGCCTCGTC-3' : 2000, 1900, 1700, 1650, 1500, 1400, 1300, 1250, 1130, 940, 920, 900, 860, 820, 760, 730, 700, 630, 600, 580, 530, 480, 460, 400.
OPC-19 5' -GTTGCCAGCC-3' : 2000, 1750, 1650, 1500, 1300, 1150, 1050, 1000, 940, 860, 810, 760, 700, 650, 600, 540, 510, 440, 400, 300.

Lates mariae
OPA-07 5' -GAAACGGGTG-3' : 1300, 1250, 1150, 1100, 1000, 960, 900, 860, 840, 700, 680, 640, 620, 600, 560, 520, 470, 440, 420, 370, 310.
OPC-05 5' -GATGACCGCC-3' : 2000, 1450, 1300, 1150, 1100, 960, 840, 740, 660, 630, 580, 560, 450, 400, 350, 280.
OPC-08 5' -TGGACCGGTG-3' : 1550, 1500, 1250, 1150, 1050, 950, 850, 800, 750, 700, 600, 560, 470, 440, 420.

Appendix 2. Primary data of RAPD band profiles in sampling occassions of *Limnothrissa miodon.*

	n	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16
dry Bujumbura	20	0	12	13	4	0	4	16	20	2	8	2	8	4	0	8	11
rainy Bujumbura	29	1	28	4	12	1	19	29	10	0	13	1	3	28	25	27	0
dry Kigoma	19	0	11	11	1	0	3	0	19	0	19	12	0	0	0	0	0
rainy Kigoma	30	0	30	1	6	0	30	28	14	0	12	1	18	23	14	30	1
rainy Kalemie	17	0	15	13	4	7	0	12	9	3	7	0	0	10	4	3	0
dry Kipili	20	0	1	10	8	0	4	11	4	16	0	5	18	0	0	1	2
rainy Kipili	29	0	29	10	2	0	24	28	13	0	12	1	11	23	18	29	1
dry Mpulungu	20	0	18	9	0	0	0	9	17	4	0	0	0	8	0	0	19
rainy Mpulungu	30	0	28	14	2	1	1	16	29	16	22	2	0	24	0	24	29
dry Nsumbu	19	0	19	0	12	0	4	11	14	0	5	2	4	4	4	2	4
rainy Nsumbu	29	0	29	10	12	5	0	14	29	15	15	2	0	29	0	29	27
Total	262	1	220	95	63	14	89	174	178	56	113	28	62	153	65	153	94

X17	X18	X19	X20	X21	X22	X23	X24	X25	X26	X27	X28	X29	X30	X31	X32	X33
1	20	20	0	0	3	1	3	0	5	0	19	0	0	20	6	0
29	29	0	0	0	27	18	12	14	18	8	28	5	8	25	22	6
19	19	0	19	0	13	0	5	2	9	0	19	0	1	0	19	18
30	30	0	0	0	0	0	0	0	2	0	30	0	1	2	30	26
17	17	0	0	0	0	0	0	0	3	0	15	1	0	3	17	15
4	2	16	12	0	15	5	8	0	11	1	20	0	1	20	7	3
29	29	0	0	16	9	7	2	2	12	1	29	2	6	13	27	17
1	0	0	1	14	7	9	7	0	7	1	20	0	0	9	13	0
0	1	30	0	0	1	0	0	0	1	4	30	3	1	22	26	10
4	4	0	0	0	5	12	8	1	9	0	18	3	0	15	8	4
0	2	29	2	0	1	0	1	2	21	1	11	2	5	22	27	8
134	153	95	34	30	81	52	46	21	98	16	239	16	23	151	202	107

X34	X35	X36	X37	X38	X39	X40	X41	X42	X43	X44	X45	X46	X47	X48	X49	X50
0	0	1	0	1	20	14	18	0	17	0	16	18	20	0	14	8
2	3	0	16	12	29	27	28	1	29	18	27	28	29	1	19	27
1	1	0	3	9	19	14	17	0	18	0	16	18	19	0	19	1
0	2	1	4	11	30	29	30	1	30	0	28	30	30	0	29	30
0	1	2	0	1	17	15	17	2	17	0	17	17	17	0	17	0
1	0	0	0	1	20	15	18	0	19	0	18	19	20	0	8	16
4	7	7	15	8	29	28	27	0	27	10	28	29	29	2	12	28
0	0	0	0	1	18	17	20	1	20	0	20	20	20	0	19	0
1	1	0	11	10	30	26	30	0	20	13	29	26	29	0	19	18
0	1	0	0	4	16	9	19	2	19	0	18	18	19	1	19	4
2	15	7	9	6	9	9	29	0	25	29	29	29	29	1	16	29
11	31	18	58	64	237	203	253	7	241	70	246	252	261	5	191	161

X51	X52	X53	X54	X55	X56	X57	X58	X59	X60	X61
0	15	0	20	0	1	7	19	12	0	1
0	29	0	29	1	2	28	22	2	0	21
0	19	1	19	0	0	0	16	16	1	1
0	30	1	30	0	7	8	30	30	16	19
0	17	0	17	7	14	16	16	12	13	8
0	15	1	20	2	8	15	20	4	0	8
6	22	2	27	0	5	22	25	9	3	3
0	18	0	20	0	0	0	18	18	0	0
0	30	4	30	10	14	16	30	30	30	13
0	18	0	19	0	4	0	19	19	5	7
2	29	4	29	1	21	23	29	29	11	12
8	242	13	260	21	76	135	244	181	79	93

Appendix 3. Primary data of RAPD band profiles in sampling occassions of *Stolothrissa tanganicae*.

	n	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15
d. Bujumbura	19	2	18	19	16	17	19	1	19	17	1	1	17	19	0	19
d. Kigoma	20	2	19	20	9	16	20	0	20	20	0	0	19	20	1	20
d. Kipili	18	0	14	18	8	18	17	0	17	17	0	3	15	18	1	18
d. Mpulungu	20	12	20	20	18	18	20	6	16	19	5	2	20	20	1	20
d. Nsumbu	19	7	19	19	16	16	11	1	17	19	8	0	17	19	2	19
r. Bujumbura	23	0	7	12	8	14	10	1	17	12	2	0	14	21	0	22
r. Kigoma	30	19	30	30	26	29	30	0	30	30	4	3	29	29	0	30
r. Kalemie	29	0	20	24	14	14	24	12	29	25	1	0	0	29	1	27
r. Kipili	30	1	29	29	18	25	29	0	29	29	9	0	25	30	0	29
r. Mpulungu	22	0	17	16	16	15	20	12	18	18	0	0	8	19	0	22
r. Nsumbu	30	8	28	22	10	21	28	9	30	25	5	0	14	20	0	26
	260	51	221	229	159	203	228	42	242	231	35	9	178	244	6	252

X16	X17	X18	X19	X20	X21	X22	X23	X24	X25	X26	X27	X28	X29	X30	X31
1	19	6	10	13	4	6	15	3	13	0	14	5	8	0	19
1	20	19	0	0	16	20	20	15	4	0	10	19	20	0	18
1	18	18	0	0	5	14	17	15	7	0	5	11	13	0	15
2	20	0	11	11	3	11	18	0	20	1	20	0	8	1	19
2	19	4	0	0	10	8	18	6	15	0	17	0	5	0	19
5	23	4	22	22	1	11	12	2	3	1	6	14	2	4	18
10	30	0	0	0	30	30	30	27	6	3	25	10	3	1	29
9	29	8	25	27	1	19	18	0	0	5	0	0	19	3	22
0	29	6	18	18	12	30	30	12	6	1	23	0	5	5	12
1	22	2	16	17	10	19	16	1	1	0	14	0	0	1	20
11	27	0	0	0	16	30	30	2	2	1	2	4	0	0	24
43	256	67	102	108	108	198	224	83	77	12	136	63	83	15	215

X32	X33	X34	X35	X36	X37	X38	X39	X40	X41	X42	X43	X44	X45	X46	X47
19	3	6	0	10	5	6	13	19	0	0	0	18	0	1	2
20	4	10	0	20	13	18	0	20	0	0	0	20	0	0	11
18	5	9	0	18	15	17	0	18	0	0	0	17	1	0	1
20	1	4	0	10	20	0	20	18	0	1	2	19	4	10	10
19	1	7	0	4	19	1	18	17	0	5	0	19	2	15	17
22	11	15	3	0	23	22	18	18	0	2	0	19	0	4	9
30	3	6	9	0	30	0	30	30	0	27	0	30	2	21	14
22	7	7	0	0	29	0	25	26	1	0	0	25	5	8	4
30	20	14	14	1	30	18	30	30	0	10	0	30	1	2	17
22	8	9	0	0	22	3	22	22	0	0	0	22	0	5	7
28	2	16	7	0	30	0	4	4	3	3	12	30	6	12	29
250	65	103	33	63	236	85	180	222	4	48	14	249	21	78	121

X48	X49	X50	X51	X52	X53	X54	X55	X56	X57	X58	X59	X60
16	8	3	0	17	15	19	15	7	5	0	19	19
12	20	3	11	15	20	20	20	20	6	1	20	19
6	16	9	11	15	16	18	16	15	1	6	17	16
20	10	0	9	16	19	20	16	18	0	0	20	20
19	9	0	12	13	19	19	17	18	1	5	19	19
22	10	0	9	0	21	16	23	22	17	3	23	23
30	4	9	25	28	24	30	27	0	30	16	30	30
27	0	0	13	20	23	23	24	26	2	6	28	29
30	12	1	16	11	25	25	28	29	14	1	30	30
22	0	0	14	5	22	6	17	18	22	0	22	17
30	0	0	0	17	29	30	0	30	30	0	30	0
234	89	25	120	157	233	226	203	203	128	38	258	222

Appendix 4. Primary data of RAPD band profiles in sampling occassions of *Lates stappersi.*

	n	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15
d.Bujumbura	30	5	1	15	21	23	4	3	22	20	8	2	27	12	5	5
r.Bujumbura	30	0	0	0	0	0	9	15	17	21	13	1	13	16	16	0
dry Kigoma	30	10	8	17	28	25	18	0	2	10	25	0	0	28	2	25
dry Kipili	30	0	0	0	3	3	4	6	25	9	9	0	8	30	29	0
rainy Kipili	30	0	0	0	0	0	1	3	30	27	18	9	30	0	10	1
dry Nsumbu	30	0	0	3	2	0	0	1	21	12	2	0	12	20	17	0
r. Nsumbu	30	0	0	0	2	7	8	25	22	11	22	13	30	4	0	0
d. Mpulungu	30	0	0	0	5	8	11	22	22	22	12	0	17	12	15	0
r. Mpulungu	30	0	0	0	1	11	0	13	24	29	10	1	30	1	6	1
total	270	15	9	35	62	77	55	88	185	161	119	26	167	123	100	32

X16	X17	X18	X19	X20	X21	X22	X23	X24	X25	X26	X27	X28	X29	X30	X31
1	4	0	17	0	21	0	2	16	0	10	2	2	11	0	0
0	0	0	5	1	27	1	1	17	1	10	4	3	15	5	12
19	7	22	24	0	0	0	30	0	28	0	19	24	30	4	0
0	0	0	0	0	4	0	0	0	0	0	3	0	1	0	0
3	0	1	14	3	30	2	5	30	4	7	11	5	8	2	11
0	0	1	0	0	28	0	3	12	0	2	3	2	11	0	17
0	0	1	10	1	30	0	0	29	3	29	7	1	30	5	13
0	0	0	0	0	21	0	0	4	0	5	0	0	6	1	3
1	0	2	21	0	30	0	1	29	1	25	3	13	22	9	5
24	11	27	91	5	191	3	42	137	37	88	52	50	134	26	61

X32	X33	X34	X35	X36	X37	X38	X39	X40	X41	X42	X43	X44	X45	X46	X47
2	1	6	1	9	15	0	0	0	0	0	0	1	21	22	2
1	1	0	0	0	0	0	0	0	0	4	0	0	23	15	7
0	0	20	0	22	1	5	6	21	10	10	29	2	30	30	19
0	0	0	0	0	0	0	0	0	0	0	3	0	29	29	2
1	14	0	0	0	0	4	0	0	0	0	1	0	28	29	0
0	0	0	0	0	0	0	0	0	0	0	0	0	7	8	4
0	0	0	0	0	0	0	0	0	0	0	0	0	27	27	1
0	0	0	0	0	0	0	1	0	0	0	0	0	8	8	0
0	9	0	0	0	0	0	0	0	0	0	0	0	28	28	7
4	25	26	1	31	16	9	7	21	10	14	33	3	201	196	42

X48	X49	X50	X51	X52	X53	X54	X55	X56	X57	X58
0	9	21	17	18	10	12	10	26	21	0
4	12	11	24	22	0	13	27	21	15	4
18	7	30	28	30	22	30	14	30	22	1
14	1	28	21	30	1	12	25	30	30	0
4	0	30	29	30	0	0	28	30	30	3
4	0	6	8	8	0	1	4	6	5	12
12	4	27	26	28	0	0	28	28	6	0
4	0	9	15	12	3	0	15	21	14	0
15	13	25	25	24	7	3	29	28	29	0

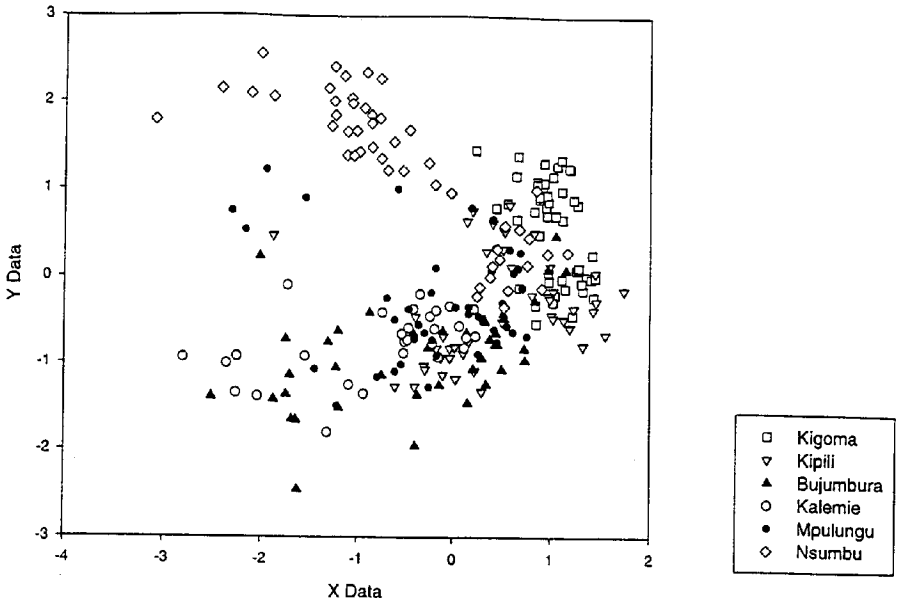
Appendix 5. Primary data of RAPD band profiles in sampling occassions of *Lates mariae*.

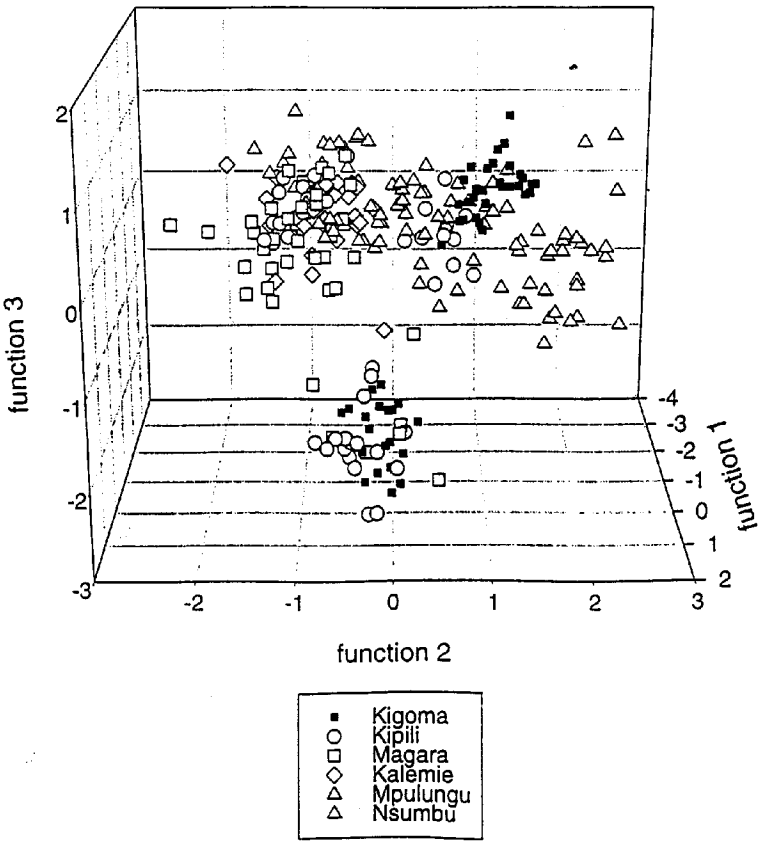
	n	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18
d.Bujumbura	23	1	22	23	0	23	0	0	23	0	0	21	0	0	23	0	23	0	0
d.Kigoma	19	0	10	10	0	10	0	0	19	0	0	12	0	0	19	0	19	0	0
d.Nsumbu	29	1	7	3	15	8	0	1	11	9	1	4	17	0	28	0	28	2	0
d.Mpulungu	30	3	29	29	30	0	19	1	1	28	0	0	23	0	12	29	11	21	5
r.Bujumbura	29	6	11	4	20	7	27	0	0	15	11	2	3	24	3	20	0	26	3
r.Kigoma	30	0	4	5	0	6	0	0	7	1	2	1	0	0	10	0	9	8	7
r.Kipili	25	21	24	0	25	5	25	1	1	25	0	2	0	14	1	3	2	0	24
r.Mpulungu	30	6	0	1	5	0	25	0	0	2	1	1	0	0	2	21	0	2	5
Total	215	37	107	75	95	59	96	3	62	80	15	43	43	38	98	73	92	59	44

X19	X20	X21	X22	X23	X24	X25	X26	X27	X28	X29	X30	X31	X32	X33	X34	X35	X36	X37
0	23	0	0	0	0	0	0	22	3	0	5	14	23	6	4	0	2	17
0	19	0	0	0	0	0	0	17	5	0	12	11	18	1	11	5	0	3
8	11	10	0	0	0	0	4	29	3	1	28	12	29	4	14	6	22	29
6	0	26	0	12	18	0	30	30	1	12	30	1	30	1	6	1	30	30
0	0	5	0	0	8	0	9	29	0	3	0	11	0	26	29	13	0	0
1	26	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	20	5	24	0	3	24	25	0	1	0	25	0	25	25	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	80	61	6	36	26	3	67	152	12	17	75	74	100	63	89	25	54	79

X38	X39	X40	X41	X42	X43	X44	X45	X46	X47	X48	X49	X50	X51	X52
1	22	0	0	23	10	23	0	23	0	0	7	13	0	0
0	8	0	14	8	13	19	0	19	5	0	6	8	5	0
0	0	0	12	18	28	28	0	29	5	0	16	21	11	0
21	30	5	27	30	30	30	12	30	2	2	21	12	17	4
0	0	0	1	28	19	26	0	28	0	0	5	1	0	0
1	1	4	5	24	1	17	23	0	25	3	1	4	7	16
16	16	4	25	0	25	25	1	25	1	0	0	0	0	0
0	4	7	13	23	28	30	1	30	5	8	29	26	18	6
39	81	20	97	154	154	198	37	184	43	13	85	85	58	26

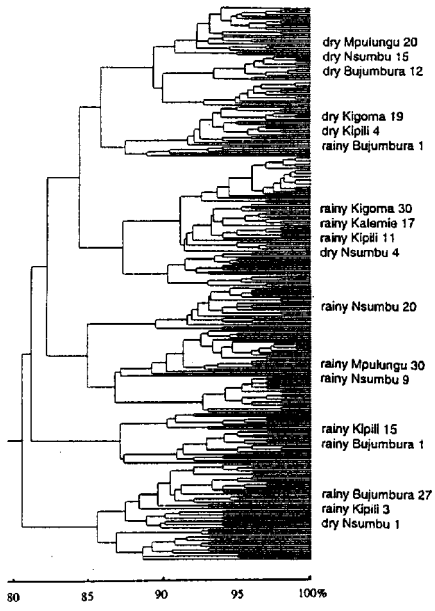
Appendix 6. Principal component analysis
Stolothrissa tanganicae



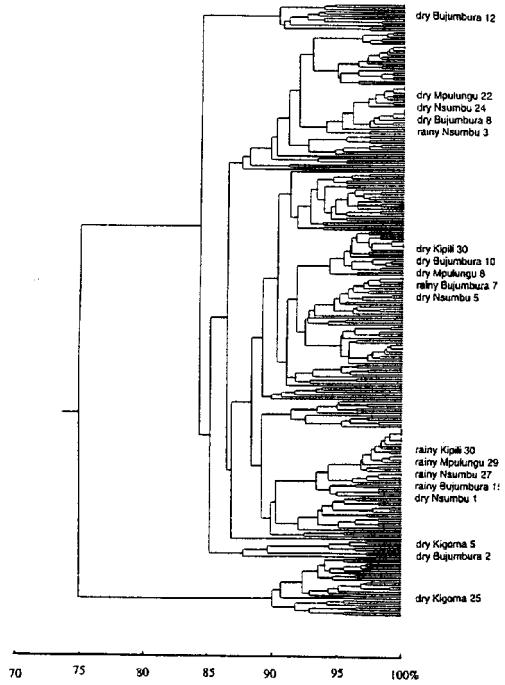


Appendix 7. Grouping of individuals based on percentual similarity of banding pattern (index M).

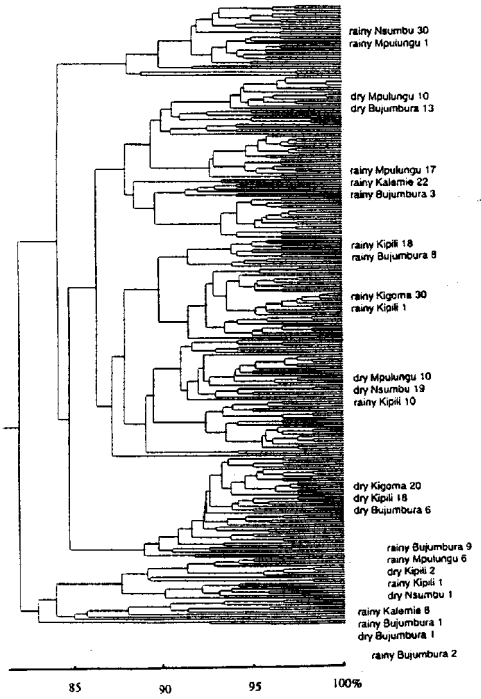
Limnothrissa miodon



Lates stappersi



Stolothrissa tanganicae



Lates mariae