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Kin cohesiveness and possible inbreeding in the mouthbrooding tilapia *Sarotherodon melanotheron* (Pisces Cichlidae)

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Abstract

Four microsatellite markers were used to study genetic variation among individuals of the mouthbrooding tilapia *Sarotherodon melanotheron* (Rüppel 1852) caught in separate but adjacent shoals. A comparison was also made with fish from six other localities. Populations originating from riverine environments appear to be panmictic, while samples from open waters such as lagoons showed highly significant heterozygote deficiencies. For instance, at the 33-allele locus SMEL4, 32 homozygous individuals were observed among the 82 individuals from the same lagoon location instead of only five homozygotes expected if random mating occurred. A further assessment of the genetic similarity of individuals within each shoal, validated by robust permutation techniques requiring no precise knowledge of gene frequencies, showed that related individuals tend to aggregate, and suggested that mating occurs preferentially within small groups of kin. Cichlids are often presented as a group of fish where microallopatric speciation takes place. The possible link between kin aggregation, inbreeding and shoaling behaviour we propose here may have important consequences for our understanding of the mechanisms involved in this fast speciation process.

Keywords: cichlids, inbreeding, kin aggregation, microsatellites, *Sarotherodon melanotheron*, tilapias

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Introduction

The shoaling behaviour of fish has recently attracted much interest (reviewed in Pitcher (1986)). Generally this trait, which may occur in reaction to environmental conditions (predation, foraging) or to favour reproduction, is thought to involve unrelated individuals (as for instance in the Atlantic cod; Herbinger *et al.* 1997). However, in a few cases, it has been proposed that the shoaling behaviour is an altruistic trait that may have evolved through kin selection. With the exception of a few studies (Ferguson & Noakes 1981; Loekle *et al.* 1982; Quinn & Busack 1985; Herbinger *et al.* 1997), there has been little direct assessment of the validity of this theory *in natura*. The existence of kin recognition has been experimentally demonstrated in salmonids (Hasler & Scholtz 1983; Quinn & Dittman 1990; Brown & Brown 1993), but the underlying mechanism (visual or chemosensory imprinting) remains an open question. Besides shoaling, kin selection might promote inbreeding, which could have important consequences for the evolution of several life-history traits, such as aggressiveness, mate choice, parental care or foraging strategies, which in turn are likely to influence the pattern of speciation. As cichlids are probably the group in which these traits have been the most thoroughly documented (reviewed in Keenleyside (1991); see also McLennan (1994)), it is interesting to investigate the extent of kin aggregation in this family.

In this study, the focus is given to the Tilapiine species, *Sarotherodon melanotheron*, in which mouthbrooding is primarily carried out by males (Pauly 1976; Trewavas 1983; Legendre & Ecoutin 1989). After the release and fertilization of several batches of eggs, the male picks them all up and carries them orally until they have developed into free-swimming fry (Keenleyside 1991). Some authors

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(for example, Trewavas (1983)) claim that brood cohesiveness, especially in tilapia mouthbrooders, is maintained beyond the stage where juveniles begin independent life.

Besides direct paternity or pedigree assessment, there are to our knowledge few studies reporting attempts to use microsatellite data to assess kinship of wild individuals for which the patterns of relatedness are unknown. There is work of this type on mice (Blouin *et al.* 1996; Garza *et al.* 1997), wombats (Taylor *et al.* 1994), bears (Paetkau *et al.* 1995), and Atlantic cod (Herbinger *et al.* 1997). In seven species of cichlids from Lake Malawi, Kellogg *et al.* (1995), looking at the progeny of 13 females, demonstrated multiple paternity. Fish are a group in which kin structure is likely to have important evolutionary consequences. Does it play a role in shoaling behaviour? This is the question we have addressed by a direct assessment of kin cohesiveness using four microsatellite DNA markers on mature fish belonging to different shoals captured in the same lagoon.

Materials and methods

Sampling

Eighty-two mature individuals of *Sarotherodon melanotheron* were caught in the Grand Lahou lagoon (Côte-d'Ivoire), belonging to eight different shoals. These shoals were visually identified in shallow water as a group of fish swimming together. All the individuals in a shoal were captured in a single netting. The different shoals were caught ≈ 10 m from each other, along a linear transect. For comparative purposes, genetic variability was assessed in six other locations: Kouilou river (n = 19; Congo), Loubi lagoon (n = 24; Congo), Bia river (n = 11; Ivory Coast), Konkouré river (n = 15; Guinée), Conakry lagoon (n = 17; Guinée), Saint Louis lagoon (n = 28; Sénégal). The details of the nettings were not available for these locations.

Recovery of microsatellite loci

A genomic library was constructed from DNA extracted from a piece of muscle taken from a freshly killed *S. melanotheron* specimen and digested with *Rsa*I, *Hae*III and *Alu*I. DNA fragments were inserted into a pUC 18 vector (*Sma*I digested and dephosphorylated); 2800 recombinant clones were obtained and transferred on to a Nylon membrane (Biodyne A). Screening was performed in hybridization bottles using radiolabelled oligonucleotidic probes. Two ^{33}P random-primed probes consisting of large poly[dG-dA]–poly[dG-dT] and poly[dG-dA]–poly[dT-dC] duplexes (Pharmacia) were used simultaneously with the same conditions as described by García de León *et al.* (1995). Three oligonucleotides (TAG)8 (TCT)8 (TGT)8 end-labelled with gamma ^{33}P-ATP were also used as described by Chenuil *et al.* (1997). Each clone presenting an insert between 300 and 600 bp was sequenced using the Sequitherm cyclic sequencing kit (Epicentre Technology) and an 'ALF' (Pharmacia) automated sequencer. Fifteen clones were used to define amplification primers and four of these oligonucleotide pairs were selected for their ability to amplify genomic DNA.

Genotype characterization and primer sequences

Chelex 100 (5%) was used for the DNA extractions from alcohol-preserved tissues (≈ 1 mg) following a standard protocol (Walsh *et al.* 1991) with proteinase K in 500 µL. One microlitre of the supernatant was used per reaction. The polymerase chain reaction (PCR) was performed in 10 µL with 0.2 units of *Tfl* polymerase (Promega), 1.5 mM $MgCl_2$, 100 µM of each dNTP, 1× reaction buffer and 10 pmol of each primer. One of the two primers was covalently linked to fluoresceine at its 5' end. The PCR programme used was: 35 min at 95 °C, 60 s at annealing temperature, 29 cycles of the three following steps, 60 s at 72 °C, 35 s at 91 °C and 35 s at annealing temperature. This cycling was followed by a 60 s elongation step at 72 °C. Annealing temperature was 53 °C for SMEL1 (GenBank number U69153), SMEL2 (X99799), SMEL5 and 50 °C for SMEL4 (X99801). The amplification products were resolved by electrophoresis on 6% polyacrylamide denaturing gels on an ALF (Pharmacia) automated sequencer.

Forward and reverse primer sequences for each microsatellite studied are (from 5' to 3'): SMEL1 (fluo-ACAAGTTGTGTAATACTGCTC and CTTTCTAATGCTTCAAAGGTG); SMEL2 (fluo-GATGCTGACTGTGACTCTAT and TCGTGTGGTTCTTGCTAAGT); SMEL4 (fluo-CAAATAATTAGTTAAATCTATG and ACTCAGTTAGACTTATCTC); SMEL5 (fluo-AGCTCCATCTCTTCAGGTTG and GGTGTGTCACAAAACCAACG).

Codominant Mendelian inheritance of the observed polymorphism was confirmed by two family studies and no mutations were detected. However, the SMEL5 primers amplify two distinct loci. The collection of observed phenotypes obtained in the family studies shows that both amplified regions are probably nonlinked and could be assigned to distinct loci. The same allelic distribution shared by the two loci does not, however, allow us to assign the origin of each allele when genealogy is unknown. For this reason, SMEL5 was treated as a tetra-allelic locus.

Data treatment

Departure from Hardy–Weinberg equilibrium

Multilocus heterozygote deficiencies were measured in the seven locations using Wright's inbreeding coefficient F, with Weir & Cockerham's (1984) estimators as implemented by the GENETIX 3.3 program package (Belkhir *et al.* 1996–1998) on all loci excluding SMEL5. In the case of the subdivided Grand Lahou sample, the average within-shoal inbreeding coefficient (in that case F_{IS}, relative to the whole sample) was also computed. The P value of multilocus F was defined as the probability of obtaining absolute values higher than or equal to the observed one under the null hypothesis (i.e. the individuals can be considered as a sample from a panmictic population at Hardy–Weinberg and linkage equilibrium). They were estimated from sets of 1000 random permutations of the original data within each population. F_{IS} in Grand Lahou was tested by permuting alleles inside shoals.

Testing the aggregation of related individuals inside shoals

In order to test for kin aggregation, we performed two separate analyses on the data. First, we asked whether or not we could identify clusters of individuals that are significantly more related to each other than to the rest of the sample. Second, we looked for evidence of nonrandom distribution of relatives within the various shoals.

Cluster assessment. There are several ways to assess relatedness from genetic data using various indexes and likelihood ratio tests (see, for instance, Queller & Goodnight (1989), Blouin *et al.* (1996), Herbinger *et al.* (1997) and Paetkau *et al.* (1995)). In order to use the information provided by all the primer pairs available (one of them not reducing to a single locus), and because estimates of allelic frequencies are very unreliable on samples of small sizes such as ours, particularly when the presence of kin structure is suspected, we used a simple classification algorithm based on allele sharing. For this purpose, a matrix showing the presence (coded 1) or absence (coded 0) of the different alleles in each individual was constructed with the data available for all loci studied and treated with the maximum parsimony criterion implemented by the MIX program of the PHYLIP 3.5 phylogeny inference software package (Felsenstein 1989), in order to identify clusters of individuals having more alleles in common. Each allele was considered as one variable and weighted by the inverse value of its frequency estimated on the eight shoals, approximated as the nearest integer on a 1–9 scale as allowed by the program. This was carried out in order to attribute more importance to the sharing of rare rather than common alleles. As often occurs with parsimony methods when they work over large and presumably homoplasic character sets, large numbers of equally parsimonious trees were produced. Therefore, we used the CONSENSE program to obtain a single majority-rule consensus tree. Only clusters showing a basal node present in 100% of the most parsimonious trees were retained for further consideration.

Clearly, such a crude technique will always produce clusters, even in purely panmictic shoals. So the validity of the groupings obtained by this approach need to be tested. We used a resampling procedure (without replacement) for this purpose. A set of 40 randomized pseudosamples, each containing eight pseudo-shoals of the same size as the real shoals, was produced using the 'permutation' procedure of the GENETIX 3.3 software, permuting over alleles within shoals for the three diploid loci and over 'genotypes' for SMEL5. Each pseudo-sample was then submitted to the same clustering procedure as described above. The resulting data were analysed by comparing the number of alleles present in a cluster as a function of cluster size in actual samples with the distribution obtained on pseudosamples. If actual clusters are made of related individuals, they should present, on average, fewer alleles than clusters produced according to the null hypothesis of panmixia.

Aggregation inside shoals. When the above step was completed, our data could be seen as a set of clusters of genetically more similar individuals distributed among our eight shoals. In order to detect nonrandom association between the two (that is to test the hypothesis that related individuals do tend to remain aggregated in the same shoal), we compared the actual distribution with a set of 40 randomized pseudosamples this time obtained by permuting the individuals over the shoals. The data produced were analysed by comparing the number of clusters as a function of shoal size in actual vs. pseudosamples. If the actual shoals are more than a random aggregation of individuals belonging to different clusters, they should contain, on average, fewer clusters than expected.

Results

Microsatellite genotyping was performed on 197 individuals belonging to seven locations for loci SMEL1, SMEL2, SMEL4. Locus SMEL5 was used only for individuals belonging to the Grand Lahou lagoon sample, this locus being treated as tetra-allelic and so only presence/absence of alleles is reported.

A summary of the observed and expected levels of genetic variation, and their significance, is given in Table 1, together with mono- and multilocus deficits (F) for all locations. The average within-shoal deficits (average F_{IS}) is also given for the Grand Lahou sample and appears to be highly significant ($F_{IS} = 0.253$, $P < 0.001$).

The three different microsatellite loci exhibited between three and 33 alleles per locus for all locations. The proportion of individuals that were heterozygous (H_O) at individual loci ranged from 0.35 to 0.73 for locus SMEL1, from 0.00 to 0.05 for locus SMEL2, and from 0.43 to 0.89 for locus SMEL4.

It can also be seen in Table 1 that Wright's multilocus fixation index *F* shows, after correction for multiple tests by a sequential Bonferroni procedure, significant values as compared with Hardy–Weinberg expectation in all but one sample located in open environments like lagoons. Conversely, nonsignificant multilocus values were found in our three samples originating from riverine systems.

The distribution of individual genotypes among loci and among shoals belonging to the Grand Lahou lagoon is given in Table 2. In Fig. 1, we give a graphical representation of the output of the maximum parsimony treatment used to define groups (or clusters) of related individuals that are supported in 100% of the most parsimonious trees. These clusters are also indicated in the second column of Table 2.

The distribution of the number of alleles per cluster according to their size is given in Fig. 2. The 577 randomized pseudo-clusters obtained from 40 pseudosamples provide the distribution under the null hypothesis of panmixia. The linear regressions for both data sets (actual and permuted) are indicated, the segment for real clusters always being below the other. According to a Fisher–Chow test of heterogeneity, this is highly significant ($P < 10^{-9}$) and indicates that the 22 real clusters contain on average fewer alleles than expected at random.

The distribution of the number of clusters of kin within eight adjacent shoals of *Sarotherodon melanotheron* according to their size is given in Fig. 3. The 40 pseudosamples provide the distribution of cluster number per shoal under the null hypothesis of no spatial aggregation of related individuals. As in Fig. 2, the regression lines for both data sets are plotted, and the line for actual values is always in a lower position. Once more, this is highly significant according to the Fisher–Chow test ($P < 10^{-6}$) which shows that the different shoals sampled in Grand Lahou contain on average fewer clusters than expected if individuals were distributed at random between shoals.

Discussion

Except for locus SMEL2, heterozygosity values were within the range found at microsatellite loci in other

Table 1 Genetic variability at three microsatellite loci in *Sarotherodon melanotheron*

		SMEL1	SMEL2	SMEL4	Multilocus
Lahou lagoon	n_{all}	7	3	33	
($n_{\mathrm{ind}} = 82$)	H_{O}	0.521	0.048	0.601	0.389
	H_{E}	0.705	0.047	0.941	0.564
	F	0.276*	−0.014 ns	0.364*	0.317*
	F_{IS}	–	–	–	0.253
Konakry lagoon	n_{all}	4	1	10	
($n_{\mathrm{ind}} = 17$)	H_{O}	0.351	0	0.761	0.371
	H_{E}	0.641	0	0.911	0.521
	F	0.456*	–	0.165*	0.286*
Saint-Louis lagoon	n_{all}	3	1	13	
($n_{\mathrm{ind}} = 30$)	H_{O}	0.367	0	0.433	0.267
	H_{E}	0.525	0	0.906	0.477
	F	0.305*	–	0.526*	0.445*
Loubi lagoon	n_{all}	3	1	14	
($n_{\mathrm{ind}} = 25$)	H_{O}	0.361	0	0.841	0.401
	H_{E}	0.422	0	0.883	0.435
	F	0.150 ns	–	0.051 ns	0.082 ns
Bia river	n_{all}	2	1	6	
($n_{\mathrm{ind}} = 11$)	H_{O}	0.727	0	0.727	0.484
	H_{E}	0.484	0	0.796	0.427
	F	0.467 ns	–	0.053 ns	−0.137 ns
Konkouré river	n_{all}	5	1	9	
($n_{\mathrm{ind}} = 15$)	H_{O}	0.733	0	0.733	0.489
	H_{E}	0.689	0	0.848	0.513
	F	−0.066 ns	–	0.134 ns*	0.048 ns
Kouilou river	n_{all}	3	1	12	
($n_{\mathrm{ind}} = 19$)	H_{O}	0.684	0	0.895	0.526
	H_{E}	0.656	0	0.879	0.512
	F	−0.045 ns	–	−0.018 ns	−0.029 ns

*Significant test after sequential Bonferroni correction.
ns, not significant.

Cichlid species studied (Kellogg *et al.* 1995; Van Oppen *et al.* 1997). Wright's multilocus fixation index F shows significant heterozygote deficiencies in all but one sample located in open environments, such as lagoons, and nonsignificant multilocus values were observed in the samples originating from riverine systems. Thus, we cannot reject the hypothesis that each of these is a random collection of individuals drawn from a panmictic population. In the only subdivided population sampled, Grand Lahou, the average within-subdivision deficit (F_{IS}) was also highly significant. It is worthwhile noting here that a similar result was obtained in an allozymic study performed on a different sampling scheme (Pouyaud 1994; unpublished), where 12 samples taken at different places and times in the Ivorian lagoon system showed a highly significant heterozygote deficiency at eight loci ($F = 0.140$, $P < 0.01$, $n = 518$), while 26 individuals of the Dodo River were at equilibrium.

Under the neutral model, heterozygote deficits are expected either when there is a mixture of different reproductive units inside the global sample ('classical' Wahlund effect), or when there is family structuring or inbreeding within the population. The existence of small groups of relatives may create small heterozygote deficiencies (or small excesses in certain cases), but if it lasts until individuals are mature, it may also promote inbreeding. Actually, in the Grand Lahou location, where our sampling scheme permitted us to separate 82 mature individuals in eight independent shoals, we found an expected heterozygosity (H_E) of 0.94 for the 33-allele locus SMEL4. In other words, we expect on average to find approximately five homozygous individuals among the 82 if random mating occurred. However, Table 2 contains 32 homozygotes. Among these homozygotes, eight individuals carried shoal-specific alleles. Grouping of noninbred relatives could not by itself produce such a high deficit of homozygotes. In shoal C (cluster 6), the shoal-specific allele SMEL4–078 was found in homozygous condition in three of six individuals, indicating that those individuals were probably themselves progeny of an inbred cross. Because their putative parents shared the same rare allele, there is a high probability that these parental individuals were kin. Other cases of shoal-specific alleles were also observed in homozygous condition in shoal C (two individuals of cluster 8 with allele SMEL4–108), in shoal D (two individuals of cluster 7 with allele SMEL4–112), in shoal B (one sample with allele SMEL4–150). Our results, thus, strongly suggest inbreeding within the Grand Lahou population (this may also be true within the other lagoon samples investigated which also show marked tendency for heterozygote deficiencies). Because we work at the microgeographic scale, it seems more parsimonious to opt for such a transient phenomenon to explain the high levels of heterozygote deficits observed, as this does not require the slow build up of significant gene frequency differences between well-identified breeding units as would be the case for the 'classical' Wahlund effect.

The next question to be asked is: are we likely to detect these groups, and if we do, how do they match with shoal composition? A visual inspection of Fig. 1 and Table 2 tends to suggest that these clusters are not randomly distributed among shoals. As explained in the section on data treatment, we tested by permutation the significance of these clusters as compared with what would happen in a panmictic population. The results presented in Fig. 2 clearly show that the actual clusters contain on average fewer alleles than clusters occurring in a random mating population, as approached by the permutation technique. We also tested the distribution of clusters among shoals. Figure 3 shows that the actual data lie below the expectation, and, thus, that the related individuals tend to remain aggregated in a limited number of clusters inside each shoal. The 10 individuals of cluster 1, all contained in shoal G, constitute a good example of this. These two results are precisely as expected under the hypotheses of aggregation of relatives eventually accompanied by a certain level of inbreeding.

Conclusion

Sarotherodon melanotheron samples originating from lagoon areas tended to show significant heterozygote deficiencies as compared with Hardy–Weinberg equilibrium expectations, whereas this was not the case for riverine samples. Despite the relatively low number of microsatellite loci available, further analysis of the Grand Lahou lagoon sample (where a microscale study could be conducted on eight different shoals caught at 10 m apart), provided evidence that these shoals were themselves composed of a nonrandom aggregation of clusters of related individuals. Some of these clusters of genetically more similar individuals strongly resembled broods of sibs or half-sibs. Furthermore, some of them probably originated from related parents, i.e. they were inbred, which would explain the high heterozygote deficit observed. This suggests that for *S. melanotheron* living in this type of environment, such as lagoons where the water hydrodynamicity is much lower than within rivers, mating often takes place within small groups of kin.

These findings have several consequences. First, the evolution of parental care has been shown to be favoured by inbreeding (Hamilton 1964; Michod 1982) and in turn to facilitate the evolution of imprinting mechanisms necessary for kin recognition (specific mating recognition system; Ribbink *et al.* 1983; Paterson 1985; Ribbink 1988). *S. melanotheron* could be a good example of this, because, as a mouthbrooder, it shows one of the most highly

Table 2 Genotypes at four microsatellite loci and cluster assignment of 82 *Sarotherodon melanotheron* individuals belonging to eight shoals caught at Grand Lahou lagoon. SMEL5 is being treated as tetra-allelic. Only the presence/absence of alleles is reported. Shoal-specific alleles are underlined

Shoal	Individual	Cluster	SMEL1	SMEL2	SMEL4	SMEL5
Shoal A	01	21	134–134	134–134	092–092	084
	02	16	137–140	134–134	092–100	066 075 084
	03	20	125–134	134–134	092–092	084
	04	5	134–137	134–134	092–152	069 084 087
	05	5	134–140	134–134	092–152	084 087
	06	5	125–140	134–134	092–152	084 087
Shoal B	07	5	134–140	134–134	092–092	069 078 081
	08	4	134–143	134–134	092–100	069 084
	14	4	134–137	134–134	100–100	069 084
	15	4	134–137	134–134	100–104	069 084
	09	15	134–134	134–134	136–138	075 081
	10	3	131–134	134–134	150–150	084 087
	11	3	128–137	134–134	114–150	084 087
	12	2	137–140	134–134	092–142	084
	13	5	131–140	134–134	092–092	066 069 078 081
Shoal C	16	6	134–134	134–134	078–078	066 084
	17	6	134–134	134–134	078–078	066 084
	18	6	134–134	134–134	078–090	066 084
	20	6	134–134	134–134	078–078	066 084 087
	21	6	134–134	134–134	078–090	066 084
	25	6	134–134	134–134	078–082	066 084 087
	19	8	137–140	134–134	108–108	75 081 084
	22	8	134–134	134–134	074–108	069 075 081
	23	8	134–134	134–134	074–108	069 081 084
	24	8	134–140	134–134	108–108	075 081 084
	26	8	137–137	122–134	108–146	069 081 084 087
	28	15	134–137	134–134	108–136	075 081 084
	29	2	131–140	134–134	122–124	084
	30	2	128–140	134–134	122–124	084
	31	2	137–137	134–134	124–124	084
Shoal D	32	22	134–134	134–134	092–092	084
	35	2	134–137	134–134	112–142	084
	33	7	134–134	132–134	112–112	081 084
	34	7	134–140	132–134	074–116	081 084
	36	7	125–140	134–134	112–112	081 084
	37	7	137–137	134–134	112–116	081 084 087
	38	7	134–140	132–134	074–112	081 084
Shoal E	39	11	134–134	134–134	096–096	075 081 087
	40	2	137–137	134–134	138–140	069 084
	41	5	140–140	134–134	092–092	081 084
	42	12	131–134	134–134	124–126	075 081 084
	43	4	134–134	134–134	114–116	069 072 084
	44	10	134–134	134–134	096–096	066 087
	45	10	134–134	134–134	096–096	066 087
Shoal F	46	3	128–131	134–134	100–116	084 087
	47	3	128–128	134–134	100–100	084
	48	3	128–137	134–134	096–104	084
	51	3	128–128	134–134	100–100	084
	54	3	128–134	134–134	100–102	087
	49	17	134–134	134–134	092–094	075 081 084
	50	18	125–134	134–134	092–092	075 084
	52	12	134–140	134–134	092–126	075 081
	56	12	131–134	134–134	124–126	066 081
	53	10	134–134	134–134	096–096	084
	55	11	134–134	134–134	080–096	075 081 084
	57	2	134–137	134–134	122–124	066 084 087

Table 2 *Continued*

Shoal	Individual	Cluster	SMEL1	SMEL2	SMEL4	SMEL5
	58	3	128–128	134–134	100–116	084
	59	19	134–134	134–134	092–092	075 084
Shoal G	60	13	134–140	134–134	094–104	084
	66	13	125–125	134–134	094–100	066 069 084
	62	14	134–137	134–134	098–100	087 090
	72	14	134–134	134–134	080–100	072 084 090
	63	1	140–140	134–134	074–100	069 084
	64	1	137–140	134–134	100–134	066 069 084
	65	1	134–140	134–134	074–100	066 069 084 087
	67	1	134–140	134–134	074–074	066 069 084
	69	1	134–134	134–134	074–134	066 084
	70	1	134–140	134–134	074–134	069 084 087
	74	1	134–134	134–134	100–134	069 084 087
	71	1	140–140	134–134	074–074	069 084
	76	1	134–140	134–134	074–074	066 084 087
	77	1	140–140	134–134	100–100	066 069 084 087
	68	9	125–125	134–134	070–084	084 087
	73	4	134–134	134–134	068–068	081 087
	61	16	137–140	134–134	116–118	069 075 084
	75	2	137–137	134–134	138–138	075 081 084
Shoal H	78	9	125–140	134–134	068–148	084
	81	9	125–134	134–134	068–148	072 084
	79	11	134–137	134–134	096–120	075 081 087
	80	4	134–134	134–134	068–068	069 084 087
	82	4	134–140	134–134	068–068	069 072 084 087
	83	2	137–137	134–134	084–138	072 084

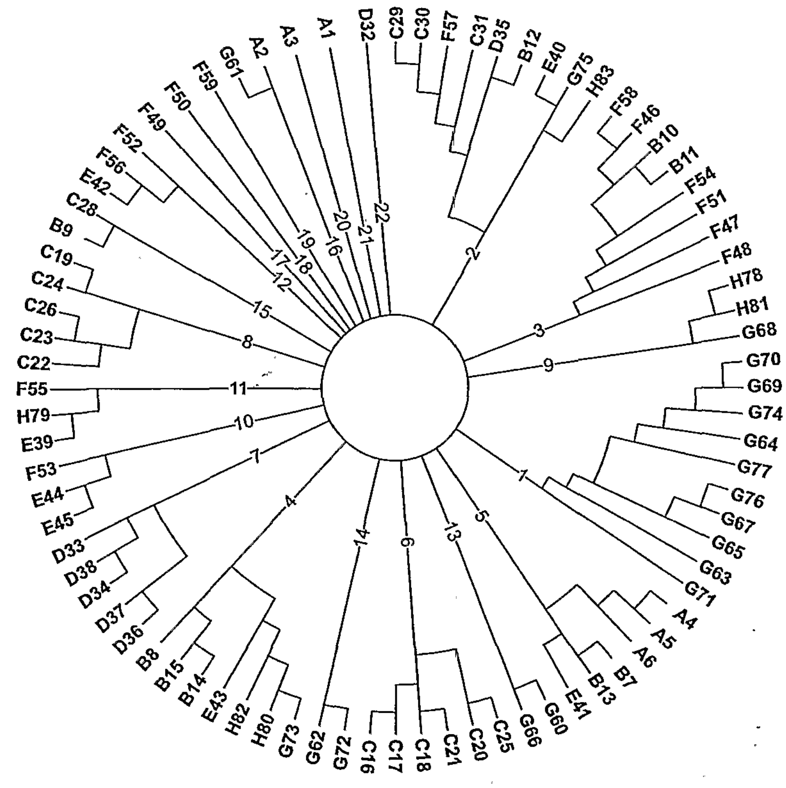

Fig. 1 Clusters of genetically related individuals inside eight adjacent shoals of *Sarotherodon melanotheron*. All the clusters shown were present in 100% of the most parsimonious trees (see text). The meaningless deeper junctions are omitted. Individuals on the outer circle are labelled as in Table 2.

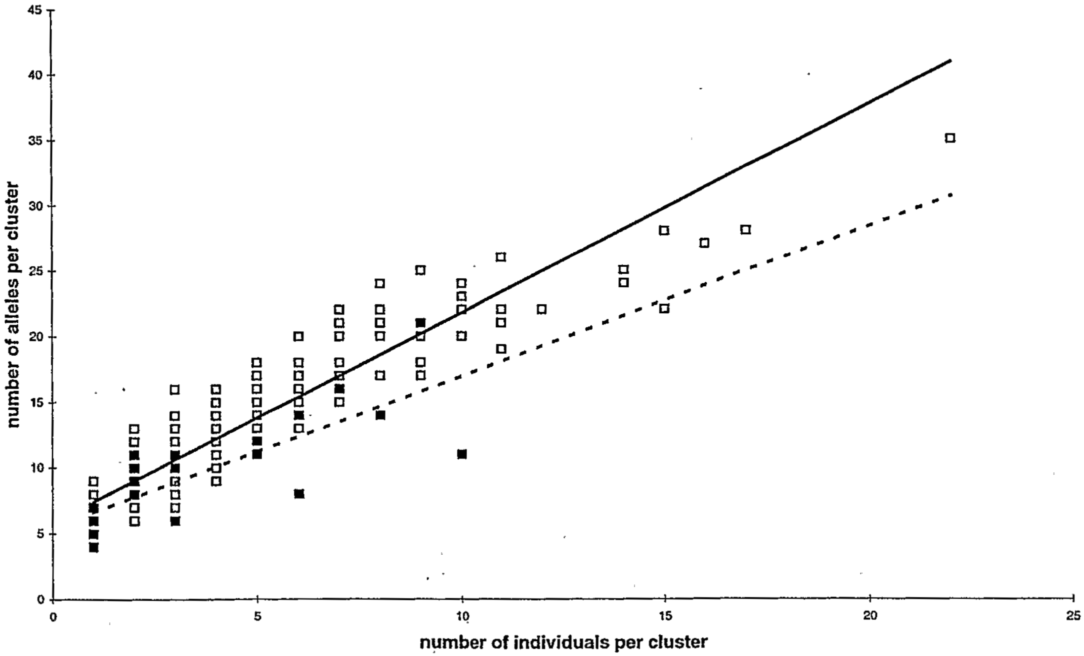

Fig. 2 Distribution of the number of alleles per cluster of kin according to their size. The white squares represent the 577 pseudo-clusters obtained by randomization on 40 pseudosamples (see text). The black squares represent the position of actual data. The upper line is the linear regression computed over permuted data and the dotted line is computed over actual clusters. The two regressions are highly significantly different. This shows that real clusters contained on average fewer alleles than expected by chance only, meaning that they were constituted of kin.

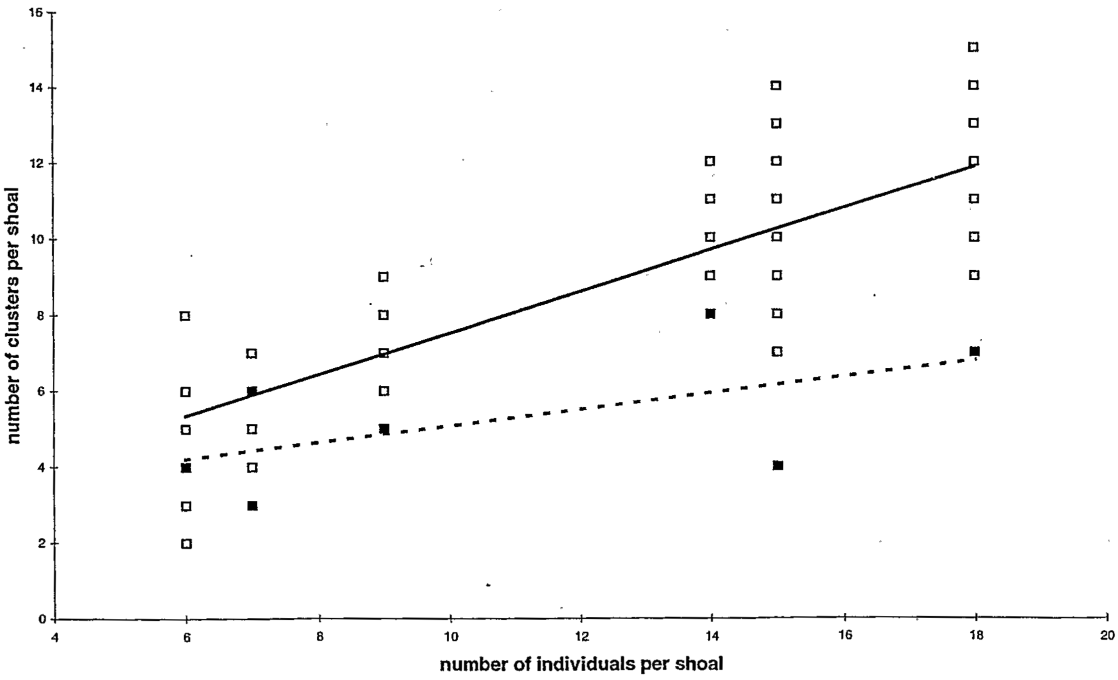

Fig. 3 Distribution of the number of clusters of kin inside eight adjacent shoals of *Sarotherodon melanotheron* according to their size. The black squares represent the position of actual shoals. The white squares represent the pseudo-shoals obtained by random permutation (see text). The upper line is the linear regression computed over permuted data and the dotted line is computed over actual clusters. The two regressions are highly significantly different, which shows that clusters of kin tended to remain aggregated and were not distributed at random among shoals.

evolved xxforms of parental care in fish (Trewavas 1983), and as reported in the present study, a noticeable level of inbreeding. Another consequence relates to the speciation patterns in the group. It has been proposed that both parental care and reproduction in small group of kin provide a powerful basis for sedentarism and sympatric or microallopatric speciation (see, for instance, Mayr (1963), Ribbink (1991), Schliewen *et al.* (1994) and Turner & Burrows (1995)). In mouthbrooder cichlids, this correlates well with the 'explosive' speciation they have undergone in the great lakes of East Africa and in the crater lakes of Cameroon (Fryer & Iles 1972; Ribbink *et al.* 1983; Trewavas 1983; Ribbink 1988; Meyer *et al.* 1990; Schliewen *et al.* 1994). The recent work of Van Oppen *et al.* (1997) on Malawi cichlids demonstrates the existence of highly significant genetic differentiation between adjacent subpopulations separated by a few hundred metres. This genetic divergence may provide opportunities for microallopatric speciation. However, we do not think that such speciation phenomena are presently taking place in *S. melanotheron*. Actually, our data support the idea that the aggregative behaviour this fish displays is probably influenced by ecological conditions such as hydrodynamic regimes (river vs. lagoon milieu), environmental pressures such as over-fishing or predation, and that in the unstable environment in which they live, this behaviour will probably produce only transient aggregations on which no long-term differentiation can build up. This putative relationship between environment and behaviour suggests, however, interesting research perspectives for the future.

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This study is part of an ongoing collaborative effort by French geneticists from ORSTOM (Institut Français de Recherche pour le Développement en Coopération) and CNRS (Centre National de la Recherche Scientifique) in order to study population genetics of tilapias in West Africa. The work was carried out in the laboratory 'Génome and Populations' managed by F. Bonhomme in Montpellier (France). Laurent Pouyaud (ORSTOM researcher) is studying molecular evolution within and among populations of Catfishes in southeast Asia. Eric Desmarais is working on phylogenetic relationships within species of the genus *Mus*. Anne Chenuil is working on the same topics on Barbels species. Jean François Agnèse (ORSTOM researcher) is interested in the phylogeography of Clariidae in Africa.

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