Evolution of *Labeo victorianus* predates the Pleistocene desiccation of Lake Victoria: evidence from mitochondrial DNA sequence variation

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Geological data show that Lake Victoria dried out some 15 000 years ago. These data suggest that the entire faunal diversity within the lake has evolved since this time. However, mitochondrial DNA sequence diversity in the endemic cyprinid fish, *Labeo victorianus*, was high (24 haplotypes in 38 individuals; percentage sequence divergence of 0.74%), suggesting that the evolution of this species predates this Late Pleistocene climatological event. This finding is consistent with what has been reported earlier for cichlid fishes in the lake.

The climatic history of Lake Victoria is reported to have fluctuated greatly in the last 400 000 years, with three major desiccations recorded from seismic studies, the most recent dating to about 15 000 years ago.[1] This has created the impression that the entire faunal diversity of the lake has arisen within the last 15 000 years. However, recent analyses based on mitochondrial DNA (mtDNA) sequences of East African cichlids have shown that the latest desiccation of Lake Victoria did not lead to a complete extinction of its endemic cichlid fauna and that the major lineage diversification took place between 100 000 and 200 000 years ago.[2–4] In this paper we report that the genetic divergence in the cyprinid fish, *Labeo victorianus,* based on mtDNA sequence variation, suggests that the evolution of this species also predates the most recent desiccation of Lake Victoria.

Samples for this study were obtained from the Kagera and Sio rivers that drain into Lake Victoria. A total of 38 samples were obtained, 19 from each river. Fresh muscle tissue was excised from under the skin, with care taken to avoid contamination, and immediately stored in 25% dimethylsulphoxide (DMSO) saturated with NaCl.[5] In the field, samples were kept at ambient temperature, and at –80°C in the laboratory. Total genomic DNA was extracted from the samples using the QIAGEN DNeasy tissue kit (QIAGEN) following the manufacturer's protocol. A 446 base-pair segment of the 5′ hypervariable part of the control region was PCR-amplified using primers *LaviF* (5′-CACCCCCT GGCTCCCAAA-3′) and *LaviR* (5′CCTCCTTGGTTTAGGGGTT TGACAAGG-3′) specifically designed for this study. The primers were biotinylated interchangeably at the 5′ end. Amplifications[6] were carried out in 50 μl reaction volumes containing 2–5 ng of total genomic DNA, 50 pmol of each primer, 50 pmol dNTPs, 10 mM Tris HCl, 1.5 mM $MgCl_2$, and 0.8 units of *Taq Polymerase* (Boehringer Mannheim). The cycling parameters used were as follows: one cycle of initial denaturation at 94°C for 5 min followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 2 min and extension at 72°C for 3 min. The double-stranded PCR product was separated into single strands using streptavidin-coated paramagnetic beads (DYNAL®). Single-stranded DNA was dissolved in sterile, distilled water and used as a template for sequencing by the dideoxy chain-termination method,[7] using Sequenase kit version 2.0 (Amersham Pharmacia Biotech), [α-^{35}S]-dATP (Amersham Pharmacia Biotech) and a primer complementary to the tem-

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plate. The products were electrophoresed in a 6% denaturating polyacrylamide – M urea gel. The gel was fixed, dried, exposed on Kodak film for 24–48 h, and read by eye.

The sequences were aligned manually using the program SeqApp[8] version 1.9. Genetic distances between haplotypes (estimated as the proportion of nucleotide differences between them), haplotype diversity, nucleotide diversity within populations and the net sequence divergence between populations[9] were estimated from the data using the program POPSTR version 1.25 (H.R. Siegismund, unpubl.). The extent of population subdivision was quantified using the F_{ST} statistic in the program ARLEQUIN[10] version 2.0, and its statistical significance assessed using 1000 random permutations.

Twenty-five segregating sites, defining 23 different haplotypes, were found among the 38 individuals sequenced (Table 1). Of these, 10 were from the Kagera River population and 10 from the Sio River samples. Three haplotypes (K964, K967 and K1028) were shared between the two populations. Sequences of these haplotypes have been submitted to GenBank (accession numbers AY839253–AY839275). The within-population number of segregating sites was high in both populations (Sio River = 19; Kagera River = 14). Only one haplotype, K965, occurred in more than one individual in the Kagera River. All other haplotypes were singletons. Uncorrected percentage sequence divergence in the total sample was 0.74%, but was 0.69% and 0.79% in Kagera and Sio river specimens, respectively. Haplotype diversity in the total sample was as high as 68%. Nucleotide diversity in the total sample (Kagera and Sio rivers combined) was 0.74 ± 0.16%. The proportion of the total genetic variation in the total sample that could be attributed to genetic differentiation between the two populations, as represented by the F_{ST} statistic, was small (–0.002) and not statistically significant ($P > 0.05$).

Table 1. Distribution of the 23 observed d-loop haplotypes from a sample of 38 *Labeo victorianus* individuals sampled from the Sio and Kagera rivers in Uganda.

Haplotype name		Segregating sites	Populations		Sum
		10 20			
		111 1111222222 23334			
		1457889055 8888000188 86793			
		9907349616 0246569356 90101	KAGERA RIVER	SIO RIVER	
1	K963*	-TC-GTATC- -TAATTCTCG ACCTT	1	–	1
2	K964	.........C C.........	5	3	8
3	K965	C........C C.........	3	–	3
4	K966	CC.......T C.T..CTA.. GT..C	1	–	1
5	K967	C......... C.........	1	1	2
6	K1002	.........C C......... GT..C	1	–	1
7	K1024	C........T C......... GT..C	1	–	1
8	K1025	...G.....C C..–.....	1	–	1
9	K1028	.... C.........	1	5	6
10	K1029	.... C.....–... GT..C	1	–	1
11	K1036	C........T C.........	1	–	1
12	K1045	.CT.AC–.TT C.T..CTA.. GTA.C	1	–	1
13	K1048	.....C.... C.........	1	–	1
14	S746	....AC...T C.........	–	1	1
15	S751	.... C......... ...A.	–	1	1
16	S957	.... C.......GC	–	1	1
17	S962	.......C.C C.........	–	1	1
18	S1030	.... C......... GT..C	–	1	1
19	S1032	.C..AC–.TT TC........	–	1	1
20	S1033	C.......TT C.T..CTA.. GT..C	–	1	1
21	S1034	.C..AC–.TT C....CTA..	–	1	1
22	S1037	.C..AC–.TT C.........	–	1	1
23	S1038	.........T C...C.....	–	1	1
Number of bases		1221221222 2221222222 22222 Sum	19	19	38

*The vertical numbers indicate the positions of the polymorphic sites relative to haplotype 1. A dash (–) represents a deletion introduced to optimize alignment.

Results of this study show that the populations of *L. victorianus* investigated in Lake Victoria harbour a high level of mtDNA sequence diversity. Indeed, the percentage sequence divergence observed in this investigation is greater than six times that reported for several cichlid fishes that inhabit the lake.[3,4,11] Mwanja[12] also reported a high level of polymorphism (63.6%) based on RAPD marker variation in *L. victorianus* populations, confirming enhanced genetic diversity in this species. In view of the mitochondrial nucleotide substitution rates reported for several of the Osteichthyes,[13,14] such a high level of polymorphism could not have arisen in only the last 15 000 years. This suggests that the evolution of the endemic *Labeo* predates the Late Pleistocene desiccation event. This interpretation is supported by recent studies of Lake Victoria cichlid fauna.[2,4,15]

We are grateful to the Fisheries Research Component of the Lake Victoria Environmental Management Project for funding this study. Stephen Buah of the Institute of Environment and Natural Resources at Makerere University is thanked for technical assistance.

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