

## Response to Comment on “Origin of the Superflock of Cichlid Fishes from Lake Victoria, East Africa”

Based on an extensive mitochondrial haplotype analysis representing about 900 specimens, we developed a conclusive model for the origin and age of the Lake Victoria (LV) superflock of haplochromine cichlids (1). Our most important findings were that (i) the cichlids were initially derived from Lake Kivu (LK), which contains the genetically most diverse and oldest cichlid lineages of any of the sampled lakes; (ii) the genetic diversity of the LV superflock must have arisen in ~100,000 years of evolution; and, hence, (iii) the LV basin might not have dried out completely, as had been suggested, but rather some (probably shallow) refuges might have existed within the basin during the late Pleistocene dry period in eastern Africa. There, a small number of lineages may have survived that maintained a good portion of the genetic variation that is found today (1). It has been debated in the paleolimnological and cichlid literature [see, for example, (2–4)] where such refugia (if any existed) might have been located and which and how many lineages of cichlids and other fish families could have survived in them.

Stager *et al.* (5) question some of the interpretations of our genetic results, based on what they perceive to be the well-documented geological history of East Africa and, in particular, of LV, which might have dried out completely ~15,000 years ago (6). They argue that no refuges at all could have existed within the LV basin, and that even if refuges had existed in LV they would have been too small, too shallow, too turbid, or chemically too concentrated to maintain a diverse set of lake-adapted haplochromine lineages. Stager *et al.* (5) further suggest that the LV cichlids evolved in parallel with the species flock of LK only during the last ~15,000 years after LV refilled, with lineages of cichlids that were seeded from potential Rwandan highland river refugial populations.

First, we point out that the geological evidence for a complete drying-out of the LV basin ~15,000 years ago may not be as unambiguous as suggested by Stager *et al.* (5). An earlier study (7) coauthored by one of the authors of the current comment concluded that “there is no evidence that the lake level fell low enough to confine fishes to refugia in small isolated ponds or around river mouths,”

and also suggested that the paleolimnological data were not sufficient to suppose that LV “dried up completely or even became highly concentrated.” Subsequent work by Johnson *et al.* (6), based on more thorough core sampling of the deepest regions of LV than in (7), suggested that the entire lake basin had indeed dried out completely. Even the data from that study, however, cannot completely rule out the presence of a few very shallow water (1 to 2 m deep) areas in the basin during the arid phase of the Late Pleistocene, just prior to 15,000 years ago (8). More recently, Fryer (2–4) has critically evaluated several recent paleolimnological studies [for example, (6, 9, 10)] and raised doubts about the geological interpretation that LV was completely dry for several thousand years in the late Pleistocene (6). Moreover, Fryer (4) analyzed in detail the paleolimnological interpretations reached by different researchers, and concluded that there is “no indication of an episode of desiccation in L. Victoria within the past 20,000 years or so.”

Regarding the suggestion of Rwandan riverine refugia (5) for the later seeding of the LV superflock during the last ~15,000 years, we have analyzed specimens from Rwandan rivers; as with all other riverine representatives of the LV superflock, these were not basal to lacustrine assemblages but were, rather, derived from them [figure 3C in (1)]. The topology of the haplotype network therefore argues against a riverine refugium. In addition, several biological arguments speak against complete LV desiccation and a riverine-refuge scenario [see also (2–4, 11)]. There are several other families of lake-adapted fishes besides the haplochromine cichlids that are only found in the LV basin today with several endemic species and even genera. Their presence would require that these families of fishes also recolonized the LV basin from rivers during the last ~15,000 years and subsequently speciated rapidly in LV—a rather unlikely scenario (4).

Stager *et al.* (5) further contend that the calibration of the molecular clock we used (12) is inaccurate, citing other molecular phylogenetic work (13) in support of their claim that the LV cichlid species flock is only ~14,000 to 16,000 years old. However, all molecular phylogenetic studies—those that were published

before (14) the paper by Johnson *et al.* (6) and, more important, those that have been published since (1, 13, 15, 16), including the reference (13) that Stager *et al.* (5) cite to the contrary—agree that the “genetic diversity [of the LV superflock] is considerably older than the 15,000 years that have passed since the lake began to refill” (13). All molecular studies suggest an age of at least 100,000 years for these genetic lineages and, probably, species, in contrast to the claim by Stager *et al.* (5). If one were willing to accept that the LV basin dried out completely ~15,000 years ago and that the genetic variation contained in the LV superflock arose only since then, a molecular clock rate 10 to 15 times faster than any previously reported for this gene for fish would have to be assumed. At present, there is no reason to accept such an exceptionally fast molecular clock rate for haplochromine cichlids. [Indeed, it is important to note that the molecular clock rate that we applied was also supported by an internal calibration that was based on undisputed geological data (1).] Whether one assumes an age of ~15,000 or ~100,000 years, the haplochromines of LV represent by far the fastest and most diverse radiation of any animal.

The apparent conflict between geological data (assuming that LV dried completely or nearly completely ~15,000 years ago) and population-genetic data (which suggest an older age for the LV species flock) may not be real. The population genetic data in our initial study (1) showed extensive haplotype sharing across species boundaries, along with large amounts of intraspecific variation. Moreover, we (1) found that Lake Kivu’s small endemic cichlid species flock is genetically more variable than the hundreds of endemic cichlids of the Lake Victoria superflock, which suggested an older age for the LK species flock than for the LV assemblage. An analysis of all previously available mitochondrial DNA (mtDNA) sequences of the first (361 bp) segment of the mitochondrial control region (1, 14, 16), along with new ones to appear in an upcoming study (17), finds a total of only 38 different mtDNA haplotypes among the hundreds of species of haplochromines in LV—an observation that underscores once again (14) the extreme degree of genetic similarity among these morphologically diverse species. The upcoming study (17) has revealed a surprisingly large number of 11 mtDNA haplotypes in 205 samples of the single haplochromine species *Xystichromis phytophagus* from the small (about 10.5 km<sup>2</sup> surface area) and shallow (less than 3 m depth in most places) Lake Kanyaboli, a satellite lake adjacent to LV in the Kenyan Yala wetlands. Thus, in this set-

## TECHNICAL COMMENT

ting, large portions of the genetic variation are manifest not between species but within them. Also, in the relatively small, shallow, and turbid lakes of the Upper and Middle Akagera in Rwanda, some 15 mostly endemic haplochromine species coexist that possess a large proportion of the morphological variability of the haplochromines from the large lakes (18).

Our finding that large amounts of genetic (and possibly morphological) diversity can be maintained in some small haplochromine cichlid species assemblages, even in small, shallow, and possibly ephemeral lakes (17), would support the hypothesis that shallow refugia might have existed during the late Pleistocene in the LV region and that surviving haplochromine lineages could have carried a good portion of the genetic diversity through the bottleneck that surely decimated the populations of most haplochromine species (1, 6). Depending on factors such as the number of surviving individuals and species and the length of the bottleneck, not much genetic variation that existed beforehand might have been lost during the last arid period in East Africa in the late Pleistocene. These results highlight the importance for further research, both paleolimnological and genetic, on this important evolutionary model system.

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19. For discussion, we thank A. Cohen, G. Fryer, T. Johnson, D. Livingstone, and P. Reinthal. Financial support came from a Fund for Scientific Research–Flanders (DWTC) project from the Belgian Federal Office for Scientific, Technical and Cultural Affairs (for E.V.) and from the Deutsche Forschungsgemeinschaft (for W.S. and A.M.).

18 February 2004; accepted 27 April 2004