Pleistocene desiccation in East Africa bottlenecked but did not extirpate the adaptive radiation of Lake Victoria haplochromine cichlid fishes

Kathryn R. Elmer[a,1], Chiara Reggio[a,1], Thierry Wirth[a,2], Erik Verheyen[b], Walter Salzburger[a,3], and Axel Meyer[a,4]

[a]Lehrstuhl für Zoologie und Evolutionsbiologie, Department of Biology, University of Konstanz, 78457 Konstanz, Germany; and [b]Vertebrate Department, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium

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The Great Lakes region of East Africa, including Lake Victoria, is the center of diversity of the mega-diverse cichlid fishes (Perciformes: Teleostei). Paleolimnological evidence indicates dramatic desiccation of this lake ca. 18,000–15,000 years ago. Consequently, the hundreds of extant endemic haplochromine species in the lake must have either evolved since then or refugia must have existed, within that lake basin or elsewhere, from which Lake Victoria was recolonized. We studied the population history of the Lake Victoria region superflock (LVRS) of haplochromine cichlids based on nuclear genetic analysis (12 microsatellite loci from 400 haplochomines) of populations from Lake Kivu, Lake Victoria, and the connected and surrounding rivers and lakes. Population genetic analyses confirmed that Lake Kivu haplochromines colonized Lake Victoria. Coalescent analyses show a 30- to 50-fold decline in the haplochromine populations of Lake Victoria, Lake Kivu, and the region ca. 18,000–15,000 years ago. We suggest that this coincides with drastic climatic and geological changes in the late Pleistocene. The most recent common ancestor of the Lake Victoria region haplochromines was estimated to have existed about 4.5 million years ago, which corresponds to the first radiation of cichlids in Lake Tanganyika and the origin of the tribe Haplochrominii. This relatively old evolutionary origin may explain the high levels of polymorphism still found in modern haplochromines. This degree of polymorphism might have acted as a ''genetic reservoir'' that permitted the explosive radiation of hundreds of haplochromines and their array of contemporary adaptive morphologies.

Bayesian statistics | haplochromine cichlids | Lake Victoria region superflock | microsatellites | population genetic structure

The species flock of more than 500 endemic species of Lake Victoria haplochromine cichlid fishes is believed to have arisen faster than any other group of species. Yet, the mechanisms of speciation by which they arose (e.g., 1–5) and the age of the adaptive radiation (e.g., 6–9) are still debated vigorously. Knowledge about both age and evolutionary history are important because the amazing variety of body shapes, assortment of coloration, behavioral diversity, and degree of ecological specialization have made African haplochromine cichlids a prime example for the study of evolution generally and adaptive radiations specifically (e.g., 10–15).

The family of cichlid fishes (Perciformes: Teleostei) is one of the most species rich groups of vertebrates, and the center of this diversity is in the Great Lakes of East Africa: Malawi, Tanganyika, and Victoria. Lake Malawi famously harbors more endemic species than any other lake in the world (16, 17), and within Lake Tanganyika, ancient cichlid lineages radiated in parallel (18–22). The Lake Victoria cichlids and those of nearby rivers and lakes Albert, Edward, George, Kivu, and Kyoga, constitute a monophyletic species flock—the Lake Victoria region 'superflock' (LVRS)—of closely related species (23, 24). Estimates from geology date the lake between 400,000 (25) and 800,000 years old (26) whereas estimates from molecular evolution date the haplochromine flock as less than 200,000 (27) or even only 100,000 years old (6). Thus, Lake Victoria is renowned for housing the fastest evolving large-scale adaptive radiation of vertebrates (12, 28, 29). The mitochondrial DNA lineages of this superflock are derived from Lake Kivu, suggesting that this relatively small, but deep and old, Rift Valley lake is the source of the present diversity of haplochromine cichlids in the Lake Victoria basin (6).

The morphological and genetic diversity of the LVRS is even more remarkable because paleolimnological data suggest a complete, or near complete, desiccation of the Lake Victoria basin between 18,000 and 15,000 years ago (25, 30, 31). If correct, the first scenario would imply that the extant haplochromine flock of 500 or more species must have evolved an order of magnitude faster than previously thought (ca. 15,000 rather than 100,000 or 200,000 years, or even longer). Alternatively, aquatic refugia could have persisted throughout the dry period and those surviving cichlids might have recolonized the re-emerging Lake Victoria (6, 28, 32) or Lake Victoria was reduced in size but did not dry out completely (6, 33, 34). Although recent mitochondrial genetic data support the latter hypothesis (5, 6, 28), geological data do not (25, 31). Consequently, the origin and age of the LVRS remain controversial (7, 35).

In this study, we used an extensive population genetic survey of haplochromine cichlids from the Lake Victoria region, along with current population genetic analytical approaches, to determine the history of the LVRS from their evolutionary origin, through the Pleistocene desiccation, up to today. We analyzed 12 microsatellite DNA loci from more than 400 cichlid specimens from Lake Victoria, Lake Kivu, and other biogeographically relevant lakes and rivers (Fig. 1). We characterized the historical and contemporary genetic diversity and demography of LVRS cichlids to test the hypotheses: (*i*) ancestral Lake Kivu or other waterbodies to the north or west founded the contemporary assemblage of haplochromines in Lake Victoria proper; (*ii*) the origin, or most recent common ancestor (MRCA), of the LVRS coincides with other dated haplochromine radiations; and (*iii*) the Lake Victoria species flock of haplochromines originated after the Pleistocene desiccation. We conclude that, in agreement with earlier studies based on mitochondrial DNA (6), Lake Victoria was colonized by Lake Kivu haplochromines. We infer that the MRCA of LVRS is old enough

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[1] K.R.E. and C.R. contributed equally to this work.

[2] Present address: Ecole Pratique des Hautes Etudes, Department of Systematics and Evolution, UMR 5202 Natural History Museum, 16, rue Buffon, 75231 Paris cedex 05, Paris, France.

[3] Present address: Zoological Institute, University of Basel, Vesalgasse 1, CH-4051 Basel, Switzerland.

[4] To whom correspondence should be addressed. E-mail: axel.meyer@uni-konstanz.de.

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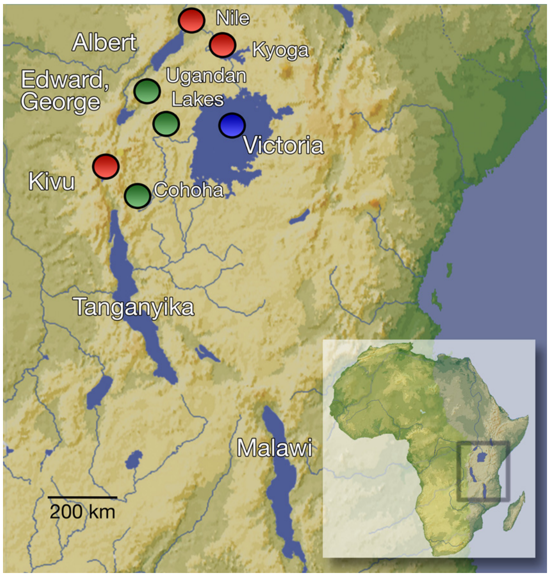

Fig. 1. The East Africa Rift Valley, showing the Great Lakes (Victoria, Tanganyika and Malawi) as well as the other waterbodies of the Lake Victoria region. Colored dots represent sampled waterbodies. Color codes for Lake Kivu (red) and Lake Victoria (blue) are used in Figs. 2 and 3.

to coincide with the primary lacustrine radiation of cichlid fishes in Lake Tanganyika, which spawned today's entire haplochromine fauna (36), and that the MRCA of haplochromines in Lake Victoria proper is almost as old. Finally, we identify that the alleles found in Lake Victoria and LVRS haplochromine cichlids greatly predate the late Pleistocene yet there is genetic evidence of a bottleneck 15,000 years ago, which we attribute to the lake's partial desiccation.

Results

The Origin of the Lake Victoria Cichlids. Several lines of evidence from microsatellite loci demonstrate that Lake Kivu is the evolutionary origin of the LVRS. First, a neighbor-joining tree analysis resolves the LVRS as monophyletic relative to the haplochromines of lakes Tanganyika, Malawi, and riverine *Astatotilapia burtoni*, and identifies the Lake Victoria haplochromines as sister taxa to, and derived from, those of Lake Kivu (Fig. 2*A*). Second, and based on the same combined data set, Bayesian cluster analyses reveal that LVRS form a single group (i.e. its constituent members are most closely related) when its constituent members are most closely related, when we assume there are maximally 5 distinct but not predefined genetic groups in the data (Fig. 2*B*, $K = 5$). The outgroup species (*L. labiatus*, *Maylandia* sp., *A. burtoni*, and *P. microlepis*) constitute the remaining 4 genetic groups. When 1 additional genetic group is assumed, the Lake Kivu haplochromines become genetically distinct and leave those of Lake Victoria and the Lake Victoria region as genetically next most similar (Fig. 2*B*, $K = 6$). Consequently, populations in Lake Victoria and surrounding waterbodies are each others' closest relatives and are derived from Lake Kivu haplochromines. Third, in the cluster analyses that focus exclusively on the LVRS and include all specimens, when 2 clusters are assumed the Lake Kivu, Nile/Kyoga, and 'Ugandan lakes' haplochromines are again unambiguously assigned together (shown in red, Fig. 3, $K = 2$), while most of those from Lake Victoria resolved into a second cluster (shown in blue, Fig. 3, $K = 2$). Nevertheless, many Lake Victoria haplochromines still show a high proportion of Lake Kivu alleles in their genome. This asymmetric pattern indicates introgression or retention of ancestral Lake Kivu alleles into the Lake Victoria basin haplochromines and suggests that the Lake Kivu haplochromine fauna colonized the Lake Victoria basin. When an additional genetic partition is assumed, most 'Ugandan lakes' and Cohoha specimens resolve into a distinct cluster that is not admixed with Lake Victoria (shown in green, Fig. 3, $k = 3$). Therefore, our findings support a Lake Kivu origin of the Lake Victoria and the 'Ugandan lakes' area haplochromines, as has been suggested previously (6) instead of Lake Edward region (37).

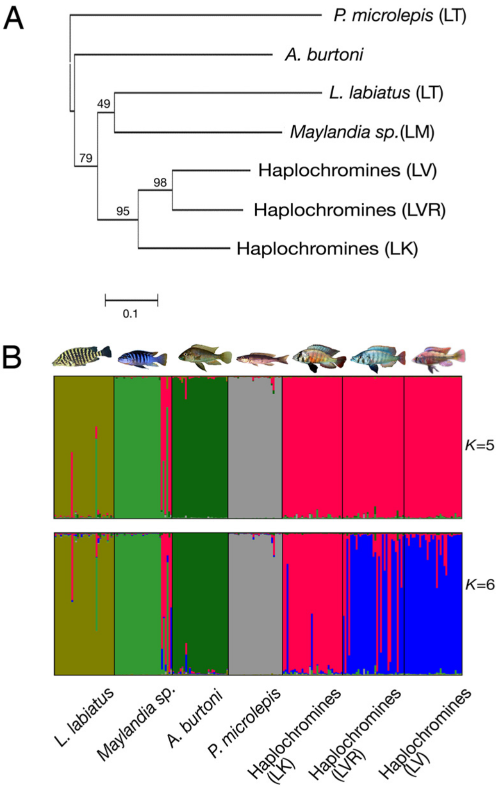

Fig. 2. Statistical genetic analyses indicate the monophyly of the Lake Victoria region superflock (LVRS) relative to cichlids from other East African lakes. (*A*) population tree based on microsatellite genotypes of the major East African haplochromine cichlid lineages included in the study: *Lobochilotes labiatus* from Lake Tanganyika (LT), the basal "Tanganyika" tribe *Perissodus microlepis* (LT), riverine *Astatotilapia burtoni*, *Maylandia* sp. from Lake Malawi (LM), and LVRS haplochromines from Lake Kivu (LK), Lake Victoria (LV), and the surrounding region (LVR). The numbers at each node are bootstrap support for the topology. (*B*) Broad level assignment tests for the same cichlid populations as above. Each vertical bar represents 1 individual and the color the proportion of its sampled genome resembling the prescribed genetic groupings. Populations of the same color are most closely related compared to the others. When the 7 populations are analyzed as 5 genetic groups ($K = 5$), the LVRS specimens form a single cluster and when 6 genetic groups are assumed ($K = 6$), Lake Kivu is separate and Lake Victoria and Lake Victoria region remain a cluster. This demonstrates that the haplochromines in Lake Victoria and the region are derived from Lake Kivu: see text.

Common Ancestors Predate the Pleistocene. We used coalescence analysis to explore the most probable demographic and genealogical histories of the LVRS. While estimating the 2 main demographic parameters of time (in generations, which here equals years) and change in population size (discussed in more detail below), time to a common ancestor is also inferred. The MRCA of the entire LVRS is estimated to have lived about 4.7 million years ago and that of Lake Victoria about 3.0 million years ago. Slightly greater ages are found for the MRCA of *A. burtoni* living in East African rivers flowing into Lake Tanganyika (3.8 million years), and for the Lake Kivu haplochromines (4.0 million years) (Table 1). Deviations around these mean age estimates are large but the

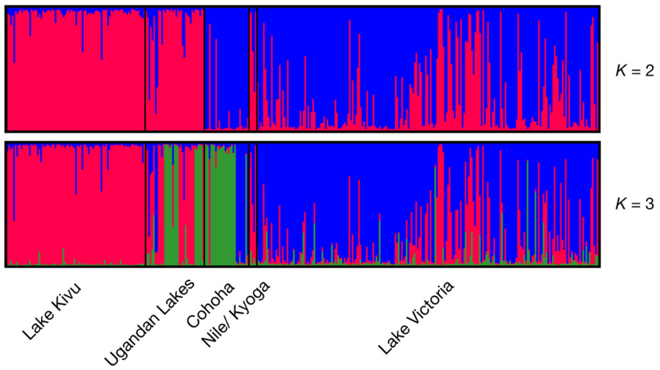

Fig. 3. Population assignment test assuming 2 or 3 genetic clusters (*K*) for all haplochromines from the LVRS. Each vertical bar represents 1 individual and its color is the proportion of its sampled genome resembling the prescribed genetic groupings. Specimens from 'Ugandan lakes' include the waterways of lakes Edward and George. When 2 genetic groups are assumed, Lake Kivu, 'Ugandan lakes', Nile/Kyoga, and a proportion of Lake Victoria individuals group together (red). Most Lake Victoria and Cohoha genomes form the second group (blue). Assuming 3 genetic groups, the 'Ugandan lakes' and Cohoha become a separate cluster (green), which is distinct from the Lake Victoria individuals. This demonstrates that Lake Victoria haplochromines are genetically most similar to those from Lake Kivu.

estimates are consistent among waterbodies. Thus, the origin of the vast haplochromine diversity in Lake Victoria far predates the presumed Pleistocene desiccation.

Population Decline Caused by Desiccation. Based on the coalescence analysis of demographic and genealogical histories, we detect a population decline ($r < 1$) (38) in all 3 assemblages of the LVRS: *N1* [the number of chromosomes (a proxy for the effective population size) at some point in time *tf*] was always found to be higher than *N0* (the effective number of chromosomes at the present time; $\theta = 2N0\mu$) (Table 2 and Table S1). The effective population size in lakes Victoria and Kivu has dropped to 3%, and in the region to less than 2%, of its previous size (present size compared to previous size; $r = N_0/N_1$). The value *ta* corresponds to the time, in generations, from when the decline (or expansion) started. In the less than 100-m deep Lake Victoria, the decline of the haplochromine assemblage started approximately 18,000 years ago; that of the 650-m deep Lake Kivu approximately 15,000 years ago; and that of the species of the other lakes and rivers approximately 17,000 years ago. Therefore, none of these haplochromine populations has yet recovered its original allelic diversity since the dramatic desiccation events of the late Pleistocene.

Contemporary Differentiation within the LVRS. Populations and species from different waterbodies in the region have their own allelic signature (Fig. 2, see also Fig. S1 and Table S2). There is significant population differentiation between the metapopulations of Lake Victoria proper and Lake Kivu ($F_{st} = 0.035$, $P < 0.001$) and between all of the haplochromine groups from different, currently geographically isolated, waterbodies within the LVRS ($F_{st} = 0.013$ to 0.104), except between Nile/Kyoga and Lake Victoria (Table S2). However, there is only low to moderate genetic differentiation among Lake Kivu species ($F_{st} = 0.008$ to 0.065) and almost no genetic differentiation among species within Lake Victoria ($F_{st} < 0.031$) (Table S3). Our findings indicate that even a reasonable number of microsatellites is insufficient to distinguish among many LVRS species within a lake.

Discussion

The Geographic Origin of the Lake Victoria Haplochromines. The origin and evolution of the enormously species-rich flock of haplochromine cichlid fishes of Lake Victoria has been hotly debated among evolutionary biologists. If the basin of Lake Victoria dried out completely approximately 18,000 to 15,000 years ago, the extant flock must have re-evolved since the basin refilled 15,000 years ago (25, 30, 31). Such a scenario is difficult to reconcile with the high number of endemic species in the lake (see, for example, 37 versus 34) and contrasts with previous genetic studies claiming that the LVRS is much older than the late Pleistocene desiccation (6, 27, 28, 32, 39, 40). The discrepancy between LVRS age estimates by population geneticists and paleolimnologists has been puzzling (31). Hence our objective was to provide data to the debate, which would allow us to describe the origin and demographic history of the LVRS in the context of the region's geology and paleoclimate.

The results of the present study confirm that Lake Kivu haplochromines acted as the source from which today's Lake Victoria basin haplochromines were colonized. The population origins inferred from nuclear genetic diversity and differentiation are highly similar to those based on previously published mtDNA (6). They confirm the clear, although recent, separation of the haplochromines of lakes Kivu and Victoria (Figs. 2 and 3, and Table S2 and reported F_{st} values). Specimens from the Cohoha area (west of Lake Victoria) are more closely related to the Lake Victoria haplochromines than to those of Lake Kivu ($K = 2$). The 'Ugandan lakes' haplochromines (including those from Lake Edward and associated waterbodies) demonstrate a genetic signature most similar to Lake Kivu (at $K = 2$) and the Cohoha region ($K = 3$), but display very little allele sharing with Lake Victoria (Fig. 3). This suggests that Lake Victoria was not colonized from the Cohoha region or Lake Edward. Moreover, while the Lake Kivu genetic assemblage is well-defined, a considerable number of Lake Victoria specimens contain a large portion of alleles that are derived from the Lake Kivu gene pool. These lakes are not currently connected but they have been in the past (41). Our data suggest an introgression of Lake Kivu genes into the Lake Victoria basin gene pool, and not the reverse, reflecting the historical direction of migration. This is concordant with geological evidence about the direction of water flow in the mid to late Pleistocene (26, 42) and the subsequent isolation due to volcanic activity (41) and reduced water stands in the Holocene (42).

Equally significant is that, although Lake Kivu currently contains only 15 endemic haplochromine cichlid species (43), it contains much more interspecific genetic differentiation than the derived and younger (although larger and much more

Table 1. Summary statistics and overall frequencies for the time to Most Recent Common Ancestor (MRCA) values for three haplochromine populations of the LVRS and one population of *A. burtoni* sampled in Kalambo River flowing into Lake Tanganyika

Species	Mean MRCA	± s.d	Median	0.025 Quantile	0.975 Quantile
Lake Victoria Haplochromines	3,045,000	1,375,000	2,975,000	2,268,000	3,896,000
Lake Victoria Region Haplochromines	4,667,000	2,248,000	4,797,000	3,196,000	6,281,000
Lake Kivu Haplochromines	3,980,000	2,060,000	4,083,000	2,538,000	5,221,000
Astatotilapia burtoni River	3,784,000	2,686,000	3,205,000	1,547,000	5,614,000

Table 2. Summary statistics of coalescent values indicating population decline based on microsatellite genotypes of three haplochromine populations from the LVRS

	Mean θ	Mean r	t_f	N_0	N_1	t_a
Lake Victoria Haplochromines	19.90	0.035	0.91	19,900	569,000	18,100
Lake Victoria Region Haplochromines	10.47	0.016	1.62	10,500	551,000	17,000
Lake Kivu Haplochromines	10.00	0.027	1.50	10,000	370,000	15,000

tf is a measure of time in generations equal to t_a/N_0, r is defined as N0/N1, where *N*0 is the present effective number of chromosomes (2Ne), and *N*1 is the number of chromosomes at time t_f, t_a is the number of generations elapsed since the decline began.

species-rich) Lake Victoria assemblage (Table S3) attesting to the much greater age of the Lake Kivu haplochromine fauna.

Our analyses confirm that the Lake Victoria haplochromines are part of a monophyletic superflock that also includes species from neighbouring lakes Albert, Edward/George, Kivu, and Kyoga as well as riverine representatives from the area (Fig. 2). This finding is supported by the relative genetic uniformity of the entire LVRS compared with all other taxa in the area (Fig. 2). Only when we partition the genetic variation further (Fig. 2*B*, $K = 6$) are members of the superflock assigned to separate groups, consistently revealing a distinct genetic structure, yet recent shared ancestry, of Lake Kivu and Lake Victoria haplochromines. This result concurs with previous biogeographic (34) and mitochondrial DNA genetic analysis (6, 27, 28, 39), which suggested that the LVRS has a common ancestor. Our results thus provide support for the out-of-Kivu hypothesis (6) for the origin of the LVRS. The various layers of agreement between mitochondrial sequences (6), nuclear genotypes, and analytical methods add credence to the approaches taken in this study.

The LVRS Is as Old as Other East African Cichlid Lineages. A major conclusion of this study is that the levels of genetic polymorphism and age of lineage coalescence that we have identified are not compatible with the species flock having arisen in situ since Lake Victoria's last major desiccation. The MRCA of the LVRS, inferred from nuclear alleles, is estimated to have lived about 4 million years ago. Similar ages for the MRCA were calculated for the assemblages of Lake Kivu and Lake Victoria, and even within *A. burtoni* from a river flowing into Lake Tanganyika (Table 2). Our data do not allow us to pinpoint the exact geographical location of the LVRS ancestor, but strongly suggest that it gave rise to the haplochromines not only of Lake Victoria but of the entire region (Figs. 2 and 3). This indicates that the colonization of Lake Victoria occurred from Lake Kivu and that the haplochomines that survived the presumably incomplete dessication of Lake Victoria must have seeded the present species assemblage within this lake. The high amount of genetic diversity we find within several species confirms the large effective population size that must have existed during the evolutionary histories of the different cichlid lineages in East Africa (also suggested based on mtDNA by references 39, 44). An alternative proposal, that cichlids have extremely high mutation rates, seems unlikely as this would require an increase in mutation rate by 2 orders of magnitude and such an anomaly is not found in any other cichlids (as suggested by ref. 34).

This estimated age of the MRCA coincides with the primary lacustrine radiation of cichlid fishes in Lake Tanganyika where the ancestor of the extant haplochromines fauna evolved (22, 36), and is slightly younger than the estimates for the MRCA of all haplochromines based on single markers (≈5 million years ago, ref. 9). During, and subsequent to, the haplochromine dispersal "out of Tanganyika" (36) it appears that high levels of polymorphism must have been maintained within the ancestral stocks, allowing them to colonize and exploit the newly developing, young river and lake environments in East Africa. In fact, we find that most of the genetic polymorphism still found in the endemic cichlids of Lake Victoria was already present in the ancestors of the modern haplochromine lineages. The discovery of ancient and substantial genetic variation may provide an explanation for the speed of the explosive adaptive radiations that characterize all of the haplochromine flocks in East Africa.

The origin of the LVRS is older than the East African desiccation but there is substantial discrepancy in the time to MRCA found between mtDNA (≈100,000 years, ref. 6) and our estimate based on nuclear markers (≈4 million years). That our analyses involve many loci and that there is concordance among the various geographical subsets of samples supports our current findings. Although mtDNA is expected to coalesce faster than nuclear alleles because of its smaller effective population size (45), the difference between mtDNA and microsatellite time to MRCA estimates suggests that other factors may also be influencing the discrepancy. These may include non-neutrality of mtDNA (46), population bottlenecks affecting mtDNA more dramatically than they do nuclear loci (47, 48), the influence of polygyny on population genetic structure, and an asymmetrical influence of a hybrid swarm on genetic diversity (49). We suggest that mitochondrial markers, being more sensitive to bottlenecks, may reflect the colonization of Lake Victoria while microsatellites, being more numerous and having broader inheritance, may reflect the expansion of all modern haplochromines.

Population Change in the Late Pleistocene. We have identified a genetic signature of the late Pleistocene desiccation: population sizes of LVRS cichlids started to decline approximately 18,000 years ago (Table 2). Throughout the history of all of the 3 African Great Lakes, climatic or geological changes influenced lake in- or out-flow patterns and caused fluctuations in water levels (50–53), including the partial, or complete, desiccation of Lake Victoria approximately 18,000–15,000 years ago (25, 30, 54). We have identified a bottleneck event that concurs with these expectations of paleoclimate change, geological evidence that Lake Victoria did not dry out completely in the late Pleistocene (42), and can explain the low mtDNA diversity and missing intermediate haplotypes of the LVRS (6) relative to the high polymorphism in nuclear markers (this study and ref. 55). Additionally, this population size flux may be a factor that promoted diversification in cichlids (44).

Population Structure within the LVRS. The number of biological species inhabiting Lake Victoria is still a matter of debate, since most still await a proper taxonomic description and a large number of species went extinct due to the recent introduction of the Nile perch and environmental degradation (56). Nevertheless, members of this radiation have diverged in morphology, ecology, and behavior to such a degree that some authors have allocated them to more than 20 different genera (23, 57). Conversely, at the molecular level, species of this assemblage display a considerable degree of shared polymorphism and to date it has been difficult or impossible to distinguish them with molecular tools (e.g., 5, 6, 28, 58, 59). Even with the rapidly evolving markers used in this study, we find an extremely low level of genetic differentiation between endemic haplochromine species and current markers and methods do not allow us to discern biological species within Lake Victoria although most species can be discerned in ancestral Lake Kivu (Table S3). Large numbers of single nucleotide polymorphisms may eventually distinguish species of the LVRS. The older cichlid adaptive radiations of lakes Tanganyika and Malawi generally contain more morphological and genetic variation than do the much younger haplochromines of Lake Victoria (60). The extensive sharing of genetic variation across LVRS species' boundaries has been attributed to: the flock's young age and the retention of ancestral polymorphism (61, 62); on-going, low levels of gene flow between species (62, 63); and/or an assemblage that may, at least in part, be due to hybridization (49). Thus, although the Lake Victoria haplochromine cichlids remain spectacular in their species richness, breadth of distinct ecological roles, and complex evolutionary history, they have not evolved in situ since the late Pleistocene.

Materials and Methods

Species and DNA Methods. This study is based on 372 haplochromine specimens from Lake Victoria, Lake Kivu, and from the lakes and rivers of the Lake Victoria region (Fig. 1 and Table S4). We also included specimens of the Tanganyika species *Lobochilotes labiatus* and *Perissodus microlepis*, of the Malawi species *Maylandia* sp., and of *Astatotilapia burtoni* (Table S4), which inhabits East African rivers as well as Lake Victoria and is phylogenetically basal. Specimen details were previously reported (6, 36, 60). We genotyped 12 nuclear DNA microsatellite loci for all specimens: OSU20D (64), TMOM11, TMOM27, TMOM5, TMOM7 (65), UNHOO1, UNHOO2 (66), ABUR25, ABUR30, ABUR162, ABUR165, and ABUR94 (67). PCR amplifications were performed according to standard protocols. The microsatellite markers were analyzed on an ABI3100 (Applied Biosystems) and scored with GENESCAN and GENOTYPER software (Applied Biosystems). These loci have been used in previous population genetic studies (e.g., 64–67) and are considered reflective of neutral patterns of evolution.

Population Genetics Analyses. Genetic differentiation between populations was measured with Wright's *F*-statistics (F_{st}) (68) based on allele frequencies and calculated in ARLEQUIN 2.01 (69). First, we determined F_{st} between the main geographically defined lineages of the LVRS. Second, we calculated genetic differentiation within each species assemblage and between those species for which sufficient numbers of individuals were available. Third, we calculated F_{st} between regional waterbodies.

The evolutionary relationship between species/species assemblages was visualized by a phylogenetic neighbor-joining tree inferred using MEGA ver. 3.1 (70) and the robustness of the branching pattern was assessed by 1,000 bootstrap replicates. Population assignment tests were conducted in STRUCTURE 2.1 (71), which is a Bayesian model-based clustering method for inferring genetic structure. STRUCTURE assumes a certain number of populations (K, where this K may be unknown), each characterized by a set of multilocus allele frequencies. Individuals are assigned probabilistically to a population, or jointly to 2 or more populations if individuals are admixed, based on maximizing equilibrium frequencies of Hardy-Weinberg and linkage (71, 72). This approach was adopted for 2 hierarchical purposes. 1) Phylogenetic context: we analyzed specimens from lakes Tanganyika (*L. labiatus* $n = 32$ and *P. microlepis* $n = 29$) and Malawi (*Maylandia* sp. $n = 31$), riverine *A. burtoni* ($n = 31$), and 32 haplochromines randomly selected from lakes Victoria and Kivu, as well as the surrounding Lake Victoria region ($n = 33$, including Cohoha, Nile/Kyoga, 'Ugandan lakes'). 2) Intrabasin clustering: we analyzed the LVRS (Lake Kivu $n = 87$, 'Ugandan lakes' $n = 38$, Cohoha $n = 26$, Nile/Kyoga $n = 6$, Lake Victoria $n = 215$) by assigning individuals to 2 or 3 genetic clusters. In both analyses, we applied the admixture model, which allows for mixed genome ancestry. Markov Chain Monte Carlo (MCMC) simulations were run with 200,000 replicates and a burn-in of 50,000.

We inferred the most probable demographic and genealogical histories based on a sample of chromosomes typed at 1 or more loci using Msvar 0.4 (38). This approach assumes a stepwise mutation model and estimates the posterior probability distributions of the genealogical and demographic parameters by MCMC simulations. The estimated parameters are scaled in terms of current population size and 2 main demographic parameters are quantified: (*i*) *tf*, which is a measure of time in generations ($= t_a/N_0$), and (*ii*) r, which is defined as N_0/N_1, where N_0 is the effective number of chromosomes (2*N*e), and N_1 is the number of chromosomes at some previous point in time t_f. For a declining population $r < 1$, for a stable population $r = 1$, and for an expanding population $r > 1$. This procedure also estimates θ, which is defined as $2N_0\mu$, where μ is the mutation rate. We assumed a generation time of 1 year (6, 73) and a mutation rate of $\mu = 5 \times 10^{-4}$ (74 and similar to 62). MCMC was run for 20,000 replicates for each population.

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