

Repeated colonization and hybridization in Lake Malawi cichlids

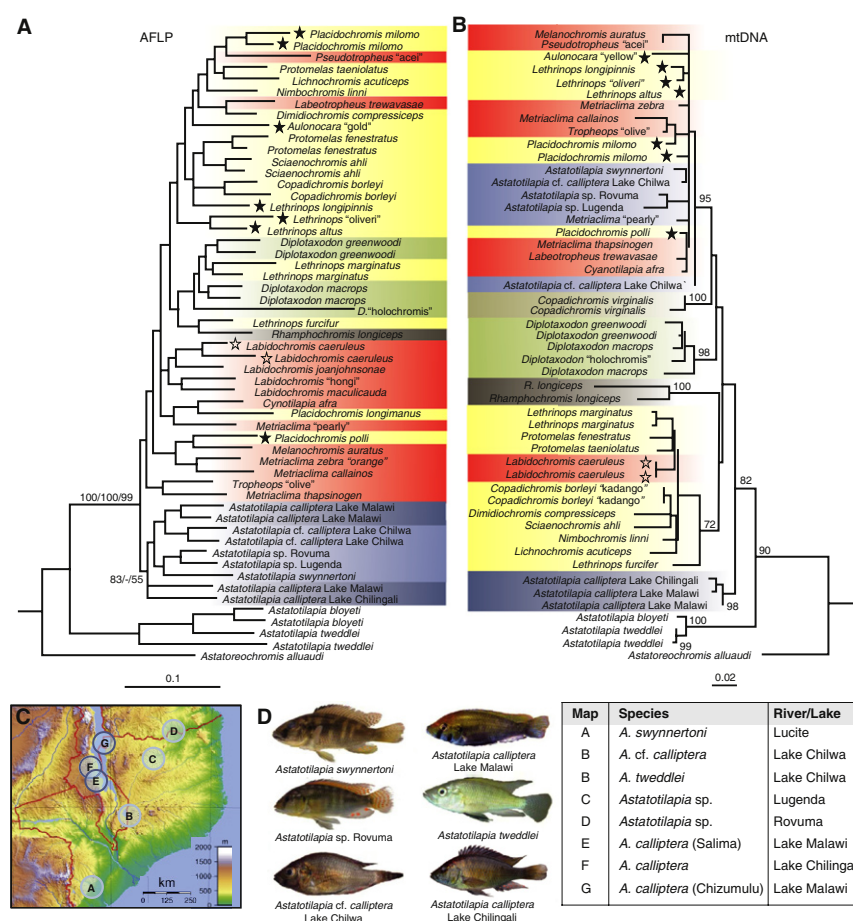
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Through adaptive radiation, ancestral species rapidly diversify into multiple species with different ecological adaptations. The haplochromine cichlid fishes of the East African Great Lakes are considered classic examples of adaptive radiation, but our understanding of the evolutionary origins of these radiations has been limited by inadequate taxonomic and genomic sampling [1,2]. Perhaps the largest of these radiations is from Lake Malawi, estimated to contain between 500 and 800 endemic species. Surprisingly, its monophyly — the origin from a single ancestral species — has never been critically tested. This is because river populations which could have seeded the radiation, with one very limited exception [3], have never been included in phylogenetic reconstructions. Moreover, phylogenies have relied heavily on mitochondrial DNA (mtDNA), which can be a misleading phylogenetic marker for species capable of hybridization [4,5] because its non-recombining nature means that transfer to other species can occur via asymmetric introgression and ‘allele surfing’ [6]. Here, we used broad taxonomic sampling and nuclear DNA markers with wide genomic coverage and find that the Lake Malawi radiation is not monophyletic, but instead contains genetic material from divergent riverine ancestors indicating multiple invasions and hybridization.

The Lake Malawi radiation consists of six deeply divergent mitochondrial lineages broadly classed as ‘mbuna’-dominated, ‘sand-dweller’-dominated (benthic), *Astatotilapia calliptera*, *Copadichromis virginalis*-dominated, *Diplotaxodon/Pallidochromis* and *Rhamphochromis* [2]. We chose taxa representing these mitochondrial clades, including species that are ecologically and morphologically

typical of each clade (for example, *Metriaclima thapsinogen* and *Cynotilapia afra* are typical mbuna) as well as atypical species such as deep water *Lethrinops* spp., which are morphologically typical sand-dwellers, but have mtDNA haplotypes that belong to the mbuna-dominated mtDNA clade. Additionally, we sampled 17 haplochromine populations from river systems inside and outside the Lake Malawi catchment (Figure 1; Supplemental information). We sequenced the mitochondrial control region (876 bp) and generated AFLP data from thirteen primer pairs. Our

analysis reveals that the Lake Malawi radiation is not monophyletic as previously thought, but contains genetic material from divergent riverine lineages which indicates multiple invasions and hybridization. The ‘mbuna-dominated’ mtDNA haplogroup was shared with an ‘eastern’ lineage of Indian Ocean drainage riverine haplochromines. This lineage includes an undescribed *Astatotilapia* from the Rovuma river, *A. swynnertoni* from the Buzi River in Mozambique, *A. cf. calliptera* from Lake Chilwa near the head of the Rovuma, and *A. calliptera* from the lower Zambezi River. *A. calliptera*



Current Biology

Figure 1. Malawi cichlid species-flock phylogeny.

(A) Phylogenetic reconstructions based on 2045 polymorphic AFLP loci using Bayesian inference (key nodes display Bayesian posterior probabilities/percentage bootstrap support for maximum parsimony/neighbour-joining). (B) Maximum likelihood analysis of mtDNA sequences with percentage bootstrap probabilities on key nodes. Further detail of phylogenetic trees and methods can be found in the Supplementary Information. Six major mitochondrial haplogroups are present in Lake Malawi; note that previously unsampled ‘eastern’ river *Astatotilapia* spp. fall in an ‘mbuna-dominated’ haplogroup. Stars highlight species morphologically discordant with their placement in the ‘mbuna-dominated’ and ‘benthic-dominated’ mtDNA haplogroups, colours indicate morphologically defined groups: blue = *Astatotilapia calliptera* spp. complex (Lake Malawi catchment = dark blue, rivers = light blue); red = morphologically mbuna; yellow = morphologically sand-dwellers (benthic); green = *Diplotaxodon*; dark green = *Copadichromis*; grey = *Rhamphochromis*. (C) Collection sites of *Astatotilapia* spp. included in analyses, outlined in the table and (D) *Astatotilapia* spp. represented.

from the Lake Malawi catchment possesses a mitochondrial haplogroup not shared with other species. Riverine taxa from the middle and upper Zambezi and the Congo drainage were unrelated to Lake Malawi taxa in both mitochondrial and nuclear genes and we found no *Astatotilapia* in those rivers. *Astatotilapia tweddlei*, from Lakes Chilwa and Chiuta and the upper Rovuma River (Indian Ocean drainage), was closely related to *A. bloyeti* from Tanzania, which together form a sister group to the Lake Malawi radiation in both mtDNA and AFLP analyses.

The nuclear AFLP phylogeny (2045 polymorphic loci) conflicted significantly with the mtDNA genealogy. The hypothesis suggested by mtDNA of five monophyletic lineages (*C. virginalis* not represented in AFLP dataset) was rejected using AFLP data (Kishino-Hasegawa test, unconstrained tree length = 5233, constrained tree length = 5386, $P < 0.001$). Similarly, the AFLP maximum parsimony topology including Lake Malawi polyphyly and *Astatotilapia* spp. monophyly was rejected in an analysis of constrained mtDNA data (Shimodaira-Hasegawa test, unconstrained tree $-\ln L = 3439.2$, constrained tree $-\ln L = 3477.8$, $P < 0.001$). Coalescent simulations of mtDNA evolution rejected the hypothesis that incomplete lineage sorting was responsible for maintenance of divergent mtDNA lineages in the nuclear clade that contained both the Lake Malawi and 'eastern' *Astatotilapia* lineages. The observed genetic distances departed significantly from those expected under an incomplete lineage sorting simulation model (minimum differences, $P = 0.007$; average differences $P = 0.016$), but did not differ from those expected under a hybridization simulation model.

Several hybridization scenarios could give rise to sharing of mtDNA between 'eastern' riverine *A. calliptera* and mbuna. One interpretation consistent with the data is that two divergent lineages of *Astatotilapia* colonized Lake Malawi. The mitochondrial signature of one invasion is still found in the Lake Malawi *A. calliptera* lineage and if *Astatotilapia* spp. originally founded the Lake Malawi species flock, this haplogroup has subsequently been lost from or remains undetectable elsewhere in the Lake Malawi flock. The second 'eastern' *Astatotilapia* mtDNA lineage survived in rivers draining into the Indian Ocean south of and including

the Rovuma and apparently colonized Lake Malawi about 0.46 million years ago (estimate from a relaxed clock accounting for time-dependent molecular evolution calibrated with dates from Gondwanan fragmentation and more recent geological events [7]), and contributed mtDNA to the species-rich mbuna lineage of the Malawi radiation. Introgression of this 'eastern' lineage with Lake Malawi cichlids formed a monophyletic nuclear clade with an *Astatotilapia*-like phenotype yet distinct Lake Malawi *A. calliptera* and 'eastern' mtDNA haplogroups ~5 million years divergent. The reproductive incompatibility clock in haplochromines ticks sufficiently slowly to allow introgressive hybridization between taxa diverged for several million years [8]. Almost all mbuna share the haplogroup with 'eastern' *Astatotilapia*, raising the possibility that hybrid populations seeded the most species-rich clade of Lake Malawi cichlids, the rock-dwelling mbuna.

Experimental work with haplochromines from Lakes Mweru and Bangweulu has shown that several million years of allopatric divergence does not prevent random mating on secondary contact when phenotypes are similar, even though mating between closely related but phenotypically divergent species is strongly assortative [9]. In Lake Malawi, hybridization events plausibly involved additional lineages, as the river *Astatotilapia*-derived and mbuna-dominated mtDNA haplogroup also contains species that phenotypically and ecologically belong to the Lake Malawi 'benthic' genera *Lethrinops* and *Placidochromis*. Conversely, the benthic-dominated mtDNA haplogroup contains *Labidochromis caeruleus*, which is morphologically mbuna.

In conclusion, a classic example of an apparently monophyletic adaptive radiation, the Lake Malawi haplochromines, is not monophyletic. It contains genomic contributions from another evolutionary lineage that survives in river systems outside the Lake Malawi catchment. Introgressive hybridization between cichlids separated for millions of years has been demonstrated experimentally, producing phenotypic novelty through transgressive segregation [10]. Our data suggest a species-rich and morphologically diverse clade of lacustrine cichlids may have originated from such introgression.

Supplemental Information

Supplemental Information including experimental procedures and two figures can be found with this article online at doi: 10.1016/j.cub.2010.11.029

Acknowledgements

We thank Walter Salzburger, Uli Schliewen, Christian Sturmbauer and Cyprian Katongo for samples. Ad Konings for picture of *A. calliptera* LM. Funded by the Leverhulme Trust grant F/00181/1 to O.S., D.H.L., G.F.T. and Swiss National Science Foundation grants 31003A-106573 and 31003A-118293 to O.S.

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