

# Cichlid fish and the micro-macroevoolution continuum in the origin of species diversity

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## Introduction

Perhaps the two most fundamental challenges to our understanding of species diversity are to explain, first, why species exist and second, why some species multiply a lot more than others. Why do some taxa undergo large evolutionary radiations, diversifying into many and varied species, while others do not? This essay is about cichlid fish diversification and what investigating it may teach us about that second question.

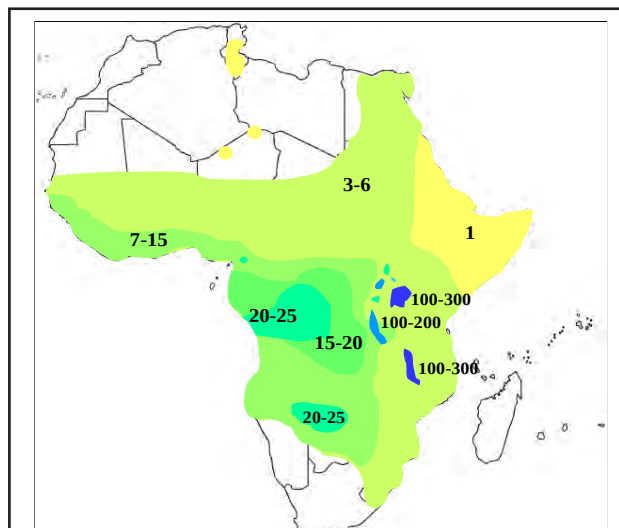
Both environmental factors such as the diversity of available resources, habitats or climates, and lineage-specific traits, such as behavioural or morphological traits, and perhaps genetic architectures, may influence the rates and extent of biological diversification. The large radiations of cichlid fishes in the African Great Lakes, Victoria, Tanganyika and Malawi, provide the most dramatic known cases of species diversification. That cichlids provide striking evidence for evolutionary stasis too and for failure to diversify is underappreciated. I argue that it is the combination of both within one taxon that makes cichlids so very suitable for addressing questions about biological diversification.

## Thirteen percent of all species of freshwater fish are cichlid fish

Cichlids are perch-like fish, distributed in freshwaters of the tropics and subtropics. With some 1300 validly described species but at least 2200 species known, they are the second most diverse family of freshwater fish after the cyprinids (2700 described species). It also is the third most species rich family of all fish, (there are 2000 described species of mostly marine gobies, Gobiidae), and probably of all vertebrate animals. At least 13% of all species of freshwater fish are cichlid fish! Cichlids have fascinated biologists and aquarist-naturalists alike for over a century because of their extraordinary diversity in morphology, ecology, colour and behaviour. Their exceptional species proliferation and accumulation of sympatric species diversity, may both be unparalleled amongst vertebrate animals.

## Cichlid fish diversity – age and distribution

Cichlid fish species richness is highly unevenly distributed in space. It is actually low everywhere except in tropical Africa, Madagascar and tropical South and Mesoamerica (table 1). Respectively, 450 and 110 species are known from tropical South- and Mesoamerica. The former number is not very high given the large area occupied in South-America, and is easily matched by several other South American freshwater fish families. In Africa and the Americas, the distribution of species richness in the cichlid fish family adheres to the latitudinal species richness gradient (Figure 1), but the exceptional species richness of cichlid fish only manifests itself in tropical Africa, where there are some 1575 known species. 1400 of these are endemic to single equatorial lakes, whereas lakes in subtropical Africa contain endemic species much less frequently, and only some 175 species are known from all the African rivers together, again predominantly in the tropical parts of the continent. In Madagascar, Meso- and South America on the other hand, most of the diversity occurs in rivers, and



**Figure 1.** The continental distribution of cichlid fish species alpha-diversity. Approximate numbers of species coexisting within area plots of 10 × 10 km (Seehausen, unpublished).

lake endemism is rare.

Molecular phylogenies suggest that cichlids are monophyletic (Streelman and Karl 1997; Sparks and Smith 2004) and that with the exception of Madagascar, cichlid faunas of major biogeographical regions also make monophyletic clades, the branching order among which is congruent with the breakup of Gondwana (Sparks 2004; Sparks and Smith 2004). If this was suggestive of a Mesozoic origin during the Late Jurassic or Early Cretaceous, the oldest known cichlid fossils, *Mahengechromis* from Tanzania and *Proterocara* from Argentina, are dated only to the Eocene (46 MYA and 33.9–5.8 MYA; (Murray 2001; Malabarba et al. 2006). Even the earliest fossils of all the labroid fish (surfperches, damselfish, wrasses and cichlid fish, now considered a polyphyletic group (Santini et al. 2009) are only about 65 MY old (Lundberg 1993).

If the earliest cichlid fossils were approximately coincident with the origin of the family, their present distribution must instead be explained by intercontinental marine dispersal (Vences et al. 2001). To resolve this conflict, Genner et al. (2007) calibrated a relaxed molecular clock with geological estimates for the ages of either Gondwanan fragmentation or cichlid fossils. Timescales of cichlid evolution derived from fossil-dated phylogenies of other bony fishes most closely matched those obtained with Gondwanan breakup calibrations, suggesting that cichlids indeed originated prior to Gondwanan landmass fragmentation 121–65 MYA. Schwarzer et al. (2009) independently confirmed the Gondwanan origin of cichlids.

Applying Gondwanan calibrations to the cichlid tree, Genner et al. (2007), Schwarzer et al. (2009) and Wagner et al. (2012) showed that accumulation of genetic diversity within many African lakes began around or after the time of lake basin formation, but also that some lakes were colonised by more than one distantly related

lineage of cichlid fish. Moreover, at least some of the large radiations are apparently derived from several such lineages that hybridised (Joyce et al. 2005; Joyce et al. 2011).

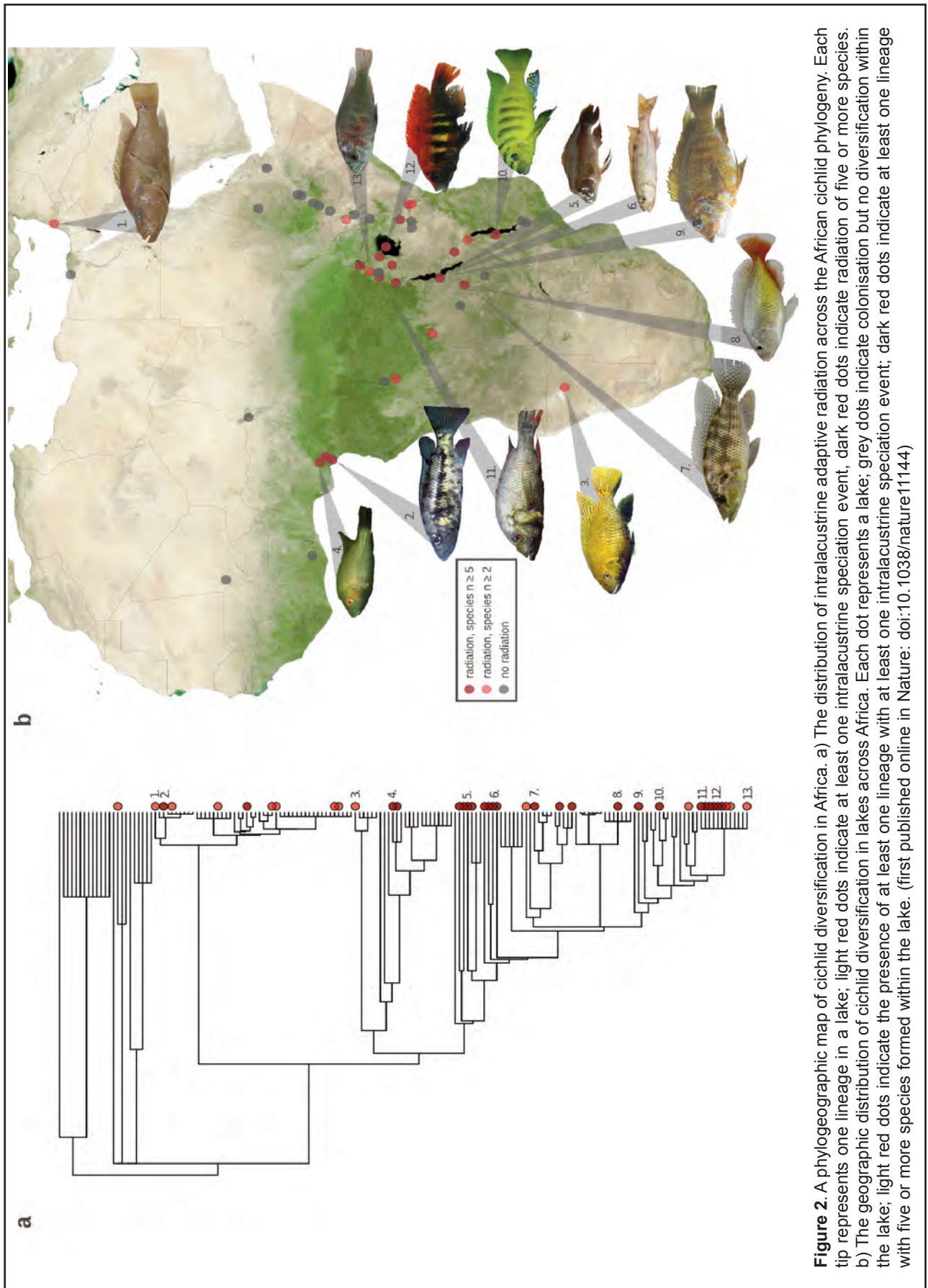
### Species are real even in the most rapid cichlid radiations

Adaptive radiation is usually thought to be associated with speciation, but the evolution of intraspecific polymorphisms without speciation could be an alternative mechanism. The cichlid fish in Lake Victoria are perhaps the most incredible example of a very recent rapid adaptive radiation, with 600+ species that arose within the past 15–100,000 years. Key questions about the rapid origin of this diversity include those about speciation versus polymorphism, whether species persist on evolutionary time scales, and whether speciation typically occurs in small isolated or in large connected populations. Studying more than 300 individuals from 105 putative species in a radiation-wide AFLP genome scan, Bezault et al. (2011) demonstrated pervasive signatures of speciation supporting the classical model of adaptive radiation. A positive relationship between the age of lakes (within the Lake Victoria region) and the average genomic differentiation of the species that occupy them, and a significant fraction of molecular variance explained by above-species level taxonomy, suggested the persistence of species on evolutionary time scales, with sequential speciation instead of a single starburst. Bezault et al. further noted that very large gene diversity was retained throughout the process to individual species, implying large effective population sizes or repeated episodes of genetic exchange. This contrasts with classical mitochondrial DNA work (e.g. Sturmbauer et al. 2001), and makes speciation in small geographical isolates highly unlikely in Lake Victoria.

Very recent population genomic investigations using next

| Area              | Number of described and valid species | Number of known species |
|-------------------|---------------------------------------|-------------------------|
| Africa            | 900                                   | 1575                    |
| Asia (South)      | 3                                     | 3                       |
| Middle East, Iran | 7                                     | 7                       |
| South America     | 290                                   | 450                     |
| Mesoamerica       | 95                                    | 110                     |
| Madagascar        | 17                                    | 32                      |
| <b>Total</b>      | <b>1310</b>                           | <b>2200</b>             |

**Table 1.** Natural distribution of cichlid fish diversity





generation sequencing of RAD tags revealed that species of Lake Victoria cichlids are phylogenetically very real entities, i.e. within a single community, all species were reciprocally monophyletic when ancestry was averaged over thousands of loci (Wagner et al. in press). Yet, gene flow between species in sympatry has sometimes been sufficient to make sympatric populations of different species more similar genetically than allopatric populations of the same species (Konijnendijk et al. 2011). Hybridisation is detectable not just as a signature of recent speciation, but hybridisation occurred between non-sister species too and apparently led to the evolution of hybrid species with novel gene combinations (Keller et al. submitted).

### Mode and mechanism of speciation

The number of speciation case studies in African lake cichlids is surprisingly small. In a series of comparative investigations of speciation, the population genetics, phenotypic differentiation and ecology of 11 pairs of geographically sympatric phenotypically defined populations of very closely related Lake Victoria cichlids have been investigated. This project wanted to explicitly compare the role in speciation of three major classes of phenotypic polymorphisms that are often associated with incipient and sometimes full speciation: male nuptial colouration, trophic (dental) morphology, and X-linked (female) colouration (Seehausen and Magalhaes 2010). Some of this work is yet to be published. The data suggest that the extent of spatial segregation of spawning sites along the sloping lake floor is a fairly good predictor of the progression stage of speciation. Such speciation, even though completely sympatric at coarse geographical scale, is ecologically parapatric at finer spatial grain.

Seehausen and Magalhaes (2010) estimated that speciation occurred even when spatial segregation accounted for just a relatively small deviation from random mating. However, of the pairs that were spatially not segregated at all, none showed any significantly differentiated allele frequencies at microsatellite loci. Several of these fully sympatric morph pairs were phenotypically nevertheless strongly differentiated. Failure to proceed in speciation could hence not be explained by lack of suitable genetic variation.

**Geographically sympatric speciation has been reported in lake-dwelling fishes more often than in any other vertebrates**

The three kinds of trait variation studied are all very common in Lake Victoria cichlids, thus one might argue that ecologically parapatric (geographically sympatric) speciation may not be uncommon in cichlid fish of Lake Victoria. Ecologically fully sympatric speciation may, though, be rare if not absent. Seehausen & Magalhaes (2010) and Konijnendijk et al. (2011) reported generally weak population differentiation at neutral markers

between conspecific allopatric populations occupying habitat islands separated by many kilometres of unsuitable habitat. This suggests that the scope for completely allopatric speciation may in fact be limited in Lake Victoria. However, there is plenty of opportunity for parapatric speciation in all its geographical facets, from allo-parapatric to para-sympatric.

The shape of the evolutionary species area relationship for African cichlid fish (Seehausen 2006) suggests a combination of more sympatric-like and more allopatric-like speciation in the evolution of cichlid species flocks. The number of endemic species has a flat relationship with lake surface area across several orders of magnitude (speciation happens even in small lakes), but suddenly increases steeply when lakes are larger than 1000km<sup>2</sup>. Hence, it seems that speciation did indeed occur in many lakes without opportunity for geographical isolation, but that many more species are produced in large lakes where there is greater opportunity for geographical genetic structure. The two arms of the curve are reminiscent of the relatively flat and steep slopes (z) respectively of intra- and interprovincial species area curves in island biogeography. There is much larger scope for allopatric speciation between provinces (or islands) than within (Rosenzweig 1997; Rosenzweig 2001). However, the increasing spatial species turnover that is expected even within a contiguous area, as sampled area exceeds species mean distribution ranges (Storch et al. 2012), can probably explain the steeper slopes at larger spatial scales. Speciation could have happened through any geographical mode.

Geographically sympatric speciation has been reported in lake-dwelling fishes more often than in any other vertebrates (Bolnick and Fitzpatrick 2007). Lakes (and the sea) differ from many terrestrial vertebrate environments (and many river environments) by the additional spatial dimension of water depth and the associated strong environmental gradients that exist within even small habitat patches. Water depth mediates gradients in light intensity and composition, oxygen concentration and temperature. These in turn affect resources, predators, parasites, sensory and signalling requirements, and hence call for divergent adaptation along the gradients. If variable depth adaptation causes deviation from random mating, traits relevant to fitness at different depth may act a little bit like “magic traits” in speciation, i.e. traits that are both under divergent natural selection and contribute directly to reproductive isolation (Servedio et al. 2011).

Our data suggest that such speciation may be typical in the rapid adaptive radiation of Lake Victoria cichlid fish. While geographically sympatric speciation may be more common in these fish than previously thought, a corollary of our observations is that spatial environmental structure does indeed have strong impact on the likelihood of ecological speciation with gene flow.

## Macroevolutionary analyses of cichlid diversification

Adaptive radiation is thought of as an evolutionary response to newly arising ecological opportunity (Simpson 1953; Losos 2010). Extrinsic factors that have been suggested to facilitate such opportunity include a paucity of competitors (Schluter 2000), predators (Vamosi 2003), or parasites (Price 2008) and biotic insularity more generally (Mac Arthur and Wilson 1967; Price 2008). Alternatively, adaptive radiation may be a consequence of the evolution of lineage-specific traits that affect speciation rates, such as key morphological innovations (Liem 1973), the prevalence of sexual selection (Kraaijeveld et al. 2011), ecological specialisation (Farrell 1998), ecological versatility (Liem 1973) and spatial vagility (Kisel and Barraclough 2010). Since the discovery of the species-rich African lake cichlid faunas, hypotheses for the spectacular diversity of these fishes have proliferated, invoking environmental factors (Fryer 1959; Sturmbauer et al. 2001), intrinsic traits (Liem 1973; Seehausen and van Alphen 1999), and their interactions (Seehausen 2007) as influences on radiation. However, at macroevolutionary scales, these hypotheses had remained untested.

Wagner et al. (2012) placed all lacustrine African cichlids from 46 different lakes on a large molecular phylogeny (Fig. 2). They collated information on species richness and endemism, lake depth, surface area, net solar radiation (hereafter “energy”), latitude, elevation, the presence of predatory fishes, and time for diversification for each lake. Then they collected data on intrinsic traits of cichlid lineages, including the presence of a polygamous mating system, mouthbrooding, generalised egg dummies and/or morphologically derived “haplochromine” egg dummies (Greenwood 1979) (used in courtship and in fertilisation of eggs in the mouth of the female), and sexual dichromatism. Using ordinary and phylogenetic multiple regression models they tested for associations between these predictor variables and species radiation – that is, whether a lineage diversified or not upon entering a lake. The best-supported predictor variables included both environmental variables and lineage-specific traits: lake depth, energy and sexual dichromatism were the most consistently well-supported predictor variables, all affecting diversification positively.

Whereas in terrestrial systems like *Anolis* lizards, where very small islands never host adaptive radiations (Losos and Schluter 2000), many of the small lakes in Africa do have little cichlid fish radiations (Wagner et al. 2012). This revealed an important difference between cichlids and terrestrial taxa in that speciation is apparently not constrained by habitat surface area in cichlids. This is also consistent with other results, discussed above, that speciation in cichlid fish may not require allopatric conditions. The consistently significant positive effect of lake depth on cichlid diversification is consistent with depth being an important axis of ecological and reproductive niche differentiation in speciation in these

fishes (Seehausen et al. 2008), providing another independent confirmation of the results of the speciation case studies. More available depth also increases habitat area for fishes, and the resultant larger population sizes may influence speciation and extinction rates. Larger energy input to an ecosystem might similarly increase carrying capacities, leading to larger total population sizes, increased rates of speciation and/or lower rates of extinction (Evans et al. 2005; Mittelbach et al. 2007). Alternatively, high inputs of energy may lead to shortened generation times and/or increased mutation rates, perhaps permitting increased rates of population differentiation and speciation. The positive effects of sexual dichromatism on diversification suggested that the intensity of sexual selection is a key influence on the probability that cichlids radiate into many species, again confirming what case studies of the microevolutionary

The coincidence between environmentally determined ecological opportunity and sexual selection best predicts whether a radiation will occur

process had revealed about the importance of sexual selection in cichlid fish speciation.

Taken together, these studies suggest that cichlid fish diversification is not a simple function of any one predictor variable, but it is the coincidence between environmentally determined ecological opportunity and sexual selection that best predicts whether a radiation will occur. Biologists often considered cichlids in general an unusually speciation-prone taxon, but because only some cichlid lineages exhibit strong sexual selection, the propensity to evolve extraordinary diversity may only have evolved in some branches of the cichlid tree. Large and deep lakes appear to play a dual major role: firstly, they capture preexisting cichlid diversity, often from several riverine sources, and subsequently maintain it sometimes well beyond its extinction in rivers, and, secondly, they are the hotspots for production of new diversity through adaptive radiation. So it seems to me that diversification may in fact be predictable in cichlid fish – but it requires taking environmental factors, evolved lineage traits and environmental history jointly into account.

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