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Primer

Adaptive radiation of cichlid fish

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How do new species arise? What is the genetic basis for adaptive morphological change and reproductive isolation? How can closely related species co-exist in the same place? What makes some groups diversify faster and more extensively than others? These are some of the questions that evolutionary biologists would like to answer by investigating the extraordinary tendency of cichlid fishes to diversify in tropical lakes. In addition, their diverse suite of mating and parental care strategies, and the importance of the tilapias in human nutrition through aquaculture, have placed cichlid fishes at the centre of an important confluence of research areas and led to the recent decision to fund the sequencing of a cichlid fish genome.

Cichlid origins and relationships
Cichlids (pronounced SICK-lids) are a family of fishes (Cichlidae) found mainly in tropical freshwaters. Mitochondrial genome sequences have indicated that cichlids are closely related to the marine surfperches (Embiotocidae) and damselfishes (Pomacentridae), but not, as previously thought, to wrasse and parrotfishes (Labridae and related families). Many fishes have pharyngeal teeth in the throat for processing their food, but fishes of all of these families have a similar and unusually structured set of pharyngeal jaws which are believed to be particularly powerful and flexible in the processing of food items. This is thought to have allowed the oral jaws to become more specialised for food capture, as they have in the cichlids and wrasse.

The natural distribution of the cichlids is centred on Africa, Latin America and Madagascar, with a few species native to south Asia and the middle east, suggesting that cichlids were already widespread throughout the great southern supercontinent Gondwana when it started to split up around 120–160 million years ago. The African cichlids have been the focus of most research, both because of their great adaptive radiations and their importance as food fish (Table 1). The African cichlids are classed into a number of 'tribes', of which the haplochromines are the most species-rich and ecologically

Table 1. Natural distribution of cichlid species.

Location	Number of known species	Estimated Age of basin (Myrs)	Major radiating lineages
African Lakes	**1,400**		
Malawi	600	8.6	Haplochromine
Victoria	>500	0.4	Haplochromine
Tanganyika	180	~20	Several
Edward	60	2.0	Haplochromine
Kivu	16	5.0	Haplochromine
Barombi Mbo	11	~1	Tilapiine
Kyoga	>10	0.4	Haplochromine
Albert	10	2.0	Haplochromine
Bermin	9	0.8	Tilapiine
Ejagham	7	0.01	Tilapiine
Mweru	6	0.35	Haplochromine
Natron	5	1.0	Tilapiine
Africa, Rest of	**175**		
Asia (South)	**3**		
Middle-East	**7**		
South America	**450**		
Mesoamerica	**110**		
Madagascar	**32**		
Total	**2,200**		

Figure 1. Examples of the diversity of shapes and colours among the haplochromine cichlids from Lake Malawi.

These species show less than 6% difference in mitochondrial DNA sequences and, within the last 5 million years, all are believed to have evolved from a single ancestor which probably resembled the swamp-dwelling *Astatotilapia calliptera* (top centre), the only one of these species presently found outside the Lake Malawi catchment.

diverse, and the tilapiines the most important in aquaculture and fisheries.

Cichlid species richness

At the last count, around 1,500 cichlid species in around 220 genera have been formally described and recognised as valid, but this is certainly a great underestimate. South American cichlid fishes are very diverse in size, morphology and behaviour and the present number of species (around 310) is probably a great underestimate, but the centres of cichlid adaptive radiation are the three largest lakes in Africa: Lakes Malawi, Tanganyika and Victoria. These lakes, particularly Victoria, have not been thoroughly sampled. Nevertheless, many species have been identified but remain undescribed, and many more species are likely to be discovered. As almost all of these species are endemic to a single lake, the total number of species in these three lakes alone could be around 1,500. Many other African lakes contain smaller numbers of endemic cichlids (Table 1) and twelve African lakes

together probably contain more than 60% of all cichlid species.

Most of the species in Lakes Malawi and Victoria are haplochromines (Figure 1). Tilapiine cichlids have diversified in other lakes, and also in Lake Malawi, but have generally produced fewer species per lake. Lake Tanganyika harbours an assemblage of very distinct cichlid lineages that may have colonised the lake independently, some with no close relatives elsewhere, and most of which have diversified to some extent. This is probably a reflection of the great age of this lake — probably 20 million years or more. Dating cichlid radiations in lakes depends on geological evidence and on molecular clocks — neither without problems. A recent recalibration of the cichlid molecular clock has suggested that their radiations tended to begin shortly after lake-basin formation. Overall, molecular phylogenetic studies have indicated that within each of the African Great Lakes, the majority of cichlid fish species have evolved much more recently than the origin of the lake and are most closely related to other

species endemic to the same lake. Thus, cichlid fishes clearly have a propensity to diversify within lake systems, with haplochromines providing the most spectacular examples.

Species and speciation

Estimates of the number of known cichlid species within a lake depend on whether colour forms are classed as species, races or morphs. Many distinct sympatric species — living in the same location — are genetically compatible and can be induced to interbreed in the laboratory, producing hybrid strains that are viable and fertile over a number of generations. In such cases, divergent mate preferences maintain reproductive isolation between the species. The species status of sympatric forms can be estimated using population genetic methods, but among allopatric forms (from different locations) it can only reliably be estimated using laboratory mate choice experiments.

Often, in the larger lakes, many cichlids occur as a series of geographically restricted colour forms, usually differing subtly in aspects of male courtship dress. Laboratory experiments have indicated that some of these forms mate assortatively, which has been interpreted as evidence that they are distinct, or at least incipient, allopatric species.

The demonstration of genetically compatible colour forms that do not interbreed in nature has led to suggestions that species may diverge as a result of divergent pathways of sexual selection leading to divergent mate preferences for courtship traits including colour. The most species-rich cichlid fishes, the haplochromines, have strongly differentiated sex roles, with all parental care provided by females — which is likely to generate strong sexual selection on male courtship traits. Cichlids also signal to potential mates using scents, sounds and movements and there is some evidence that olfactory and possibly acoustic signals may be involved in reproductive isolation. There may even be cryptic

species that look the same but sound or smell different.

Not all cichlid fish radiations involve species where strong sexual selection is likely. The cichlids of the Cameroonian lakes, such as Barombi Mbo, generally show less sex role specialisation and sexual dimorphism, and there is little differentiation in breeding colour between most species. The same is true for the Nicaraguan crater-lake species and for the species-rich lamprologine group in Lake Tanganyika. For these groups, it seems more likely that speciation has been driven by ecological rather than sexual selection and this in turn suggests that haplochromine divergence has probably been driven by a combination of both processes.

The geography of speciation
The cichlid fishes of Lake Barombi Mbo provide one of the most convincing examples of sympatric speciation in animals. Molecular phylogenetic studies, using both nuclear and mitochondrial DNA, have convincingly shown that all eleven species in this tiny lake are more closely related to each other than to any other plausible relative, and that the closest relative of the whole group is the only cichlid species found in the surrounding streams.

Suggestions of a role for sympatric speciation in other cichlid radiations have proved more controversial, and there are a number of plausible allopatric speciation scenarios in the case of the larger lakes. Over geological time, the water levels of the major lakes have risen and fallen. This has led to isolation and reconnection of populations in water bodies around the main lake, in sub-basins within the present lake boundaries or in patches of habitat within a continuous water body (Figure 2). The last option is particularly likely in the case of species specialised to patchily distributed habitats such as rocky coasts, and is consistent with observations of limited gene flow between adjacent habitat patches, and with

Figure 2. The rocky and sandy shores and the open waters of Lake Malawi provide a variety of habitats for cichlid fishes, which are heavily exploited by small-scale fisheries.

the occurrence of geographically restricted colour races that show assortative mating.

Although molecular studies have hitherto suggested that most speciation has taken place within lake catchments, interest has recently focussed on the possibility that multiple colonisations of a lake may stimulate adaptive radiation in a surprising way — through the creation of a hybrid swarm containing greater adaptive genetic variation than any of the individual founder species, allowing the generation of novel genetic combinations. There is some evidence for this theory from molecular phylogenetic studies, and indeed the potential of many cichlid species to form fertile hybrids with each other suggests that the process may continue to operate throughout the history of an adaptive radiation — even a relatively old one like in Tanganyika.

Adaptive radiation
Cichlids are celebrated for their adaptive radiation, as well as their species richness. Descended from a species that mainly sifts algae from soft sediments, the cichlid fishes of Lake Barombi Mbo include predators of fish and insects, an egg-eater, a dwarf zooplanktivore, filter-feeders and even a specialised sponge-eater. This is a small radiation of eleven species.

Lake Malawi, perhaps the largest radiation, includes a species that removes parasites from the skin of catfish, and another that bites off pieces of their skin. There are scale eaters,

fin-biters and several species that feed on eggs and larvae carried by mouthbrooding female cichlids. A guild of species inhabits empty snail shells. There are numerous species of rock-scrapers, sediment sifters, zooplankton feeders and fish eaters. There are streamlined, silvery offshore shoaling fish and their fang-toothed predators. There are specialised crab-eaters and many snail-eaters. One species feeds mainly on flies that rest on rock surfaces near the water; another turns over stones to look for hidden insect larvae.

These ecological and behavioural adaptations are associated with morphological changes to body shape and colour, head shape, jaw size, shape, orientation and musculature and to the size, shape and number of cusps of the teeth. Some species have evolved huge eyes to enable them to see in the dim light at depths of over 100 metres. Others have expanded lateral line canals to detect movements of prey hidden in the mud.

A striking feature of cichlid adaptive radiation is the tendency for very similar adaptations to appear in different lakes. There had been some debate over whether such specialised morphologies could indicate close affinities between species from different lakes, but molecular phylogenetic studies have convincingly demonstrated that they represent cases of parallel evolution.

It is less commonly mentioned that cichlids have failed to evolve some forms. In the three largest

African lakes, other fish families have filled the niches of small fast-moving plankton feeders, eel-shaped species and truly nocturnal forms. There are no really large predatory cichlids. Even the biggest African cichlid predators reach maximum sizes of only about 3–3.5 kg in weight, compared to 60–200 kg for the largest catfish and Nile Perch in the same waters. The constraints on cichlid adaptive evolution might also shed light on the causes of their diversification. Equally, insight may be gained from the considerable variation among cichlid lineages in their tendency to undergo adaptive radiation. For example, the genera *Pseudocrenilabrus* and *Tilapia* are widely distributed in Africa, yet have shown no adaptive radiation in Malawi, Tanganyika or Victoria.

Social behaviour
Cichlid fishes have complex social behaviour and all show high levels of parental care, even after the eggs have hatched. The ancestral state among cichlid fishes appears to be biparental substrate-brooding, where male and female take care of a brood of adhesive eggs laid on a stone or other hard substrate, and continue to guard the larvae and later the independently feeding fry. This is unusual among fishes.

The greatest variety of reproductive strategies is found in Lake Tanganyika. Territorial adult male *Lamprologus callipterus* are twelve times heavier than their mates — the greatest male-biased animal size ratio known. Females have to be small enough to fit inside empty snail shells where they rear their young. Dominant territorial males are large enough to pick up the shells in their mouths and their reproductive success is related to the size of their shell collection. Smaller males of this species can show alternative strategies, either mimicking females to sneak into snail shells, or else hunting for food and mates in packs. Several Tanganyika species live and breed in larger social groups, where a dominant pair produces most of the

offspring while subordinates help rear the offspring and defend the territory.

Such complex systems are known among birds and mammals, but their relatively small sizes and short generation times make cichlid fishes a suitable vertebrate model for the study of such systems. Neurobiologists have found that dominant females in such groups are masculinised in behavioural, hormonal and gene expression assays, despite having lower 11-ketotestosterone levels and being fully reproductively competent females.

Mouthbrooding is not uncommon in fishes, but maternal care is rare, yet cichlids seem to have evolved maternal mouthbrooding independently on several occasions, almost invariably from an ancestor in which both sexes showed parental care. In Lakes Malawi and Victoria, the great radiations in ecomorphology have not been accompanied by similar radiations in social behaviour — all known haplochromine cichlids are maternal mouthbrooders, suggesting that this strategy is stable irrespective of the habitat and ecology of the species. The major variation in maternal mouthbrooding is whether females subsequently also guard broods of free-swimming fry or simply release independent young.

In this system, sex roles are strongly differentiated, with males being generally larger, brighter and more aggressive. Males of some species are highly seasonal breeders that build huge and complex display structures (or 'bowers' by analogy with bower birds) from sand. Females have been shown to prefer males with particular bower characteristics, such as size and shape, and this may facilitate speciation. In other species, usually on rocky shores, males maintain permanent breeding territories in which they also obtain most of their food. Males may retain the same territory for many years and competition over breeding sites is fierce. Neurobiologists

have become interested in the changes in hormone production and gene expression associated with the switch from cryptic, shoaling subordinate to dominant sexually-active territory-holder in such species, with *Astatotilapia burtoni* becoming a favoured model.

Recently, evolutionary biologists have become increasingly aware of the possible role of aggressive behaviour in driving the differentiation of species. Aggressive interactions are generally strongest among members of the same species, which compete for a greater proportion of their critical resources, while individuals of other species may be largely ignored. As closely related species may not only compete for resources, but also produce similar signals, aggressive interactions between such species may have important evolutionary consequences. This may allow atypical individuals to avoid aggression and thus increase in frequency in the population, helping to drive sympatric speciation — assuming the atypical form is a preferred mate for part of the population.

Genetics of adaptive radiation
The large number of genetically similar, but morphologically diverse cichlid fish has been likened to a natural mutagenesis screen. With model organisms, such as zebrafish, mutant strains are generated and maintained to allow investigation of the developmental genetic basis of traits through the study of genetic defects. By contrast, closely related natural cichlid species are presumed to differ in a few genes that are responsible for major adaptive morphological traits. Many cichlid species can be hybridised in the laboratory, permitting the use of classical intraspecific methods to study the genetic bases for traits.

Quantitative trait locus (QTL) analysis of hybrids between two morphologically divergent algal scrapers from Lake Malawi has indicated that many morphological traits have a relatively simple genetic

basis — for example, a single gene is probably responsible for the difference between conical and tricuspid teeth — a trait that was once used to differentiate cichlid genera. Currently there is a lack of a good model system for the study of vertebrate dentition, and cichlids may fill this role. Using the same hybrids, a locus influencing jaw shape was mapped to the region of the gene encoding bone morphogeneic protein 4 (*bmp4*), and experimental overexpression of this gene in zebrafish led to morphological changes similar to those distinguishing the cichlid genera.

The cichlid visual system has also been the focus of much recent research, as it may play a key role in adaptation to different ecological niches as well as in speciation facilitated by preference for different-coloured mates. Cichlids have well-developed colour vision, generally operating a trichromatic system, like humans, of three cone pigments and a rod (monochrome) pigment. However, an individual may have more than three cone pigment genes, expressing a different set as succeeding life history stages occupy different habitats. Similarly, adult cone pigment sets vary between species, showing some correspondence to habitat preferences. Remarkably, there has been parallel evolution of identical protein sequences in blue-shifted rod pigments among cichlid lineages that have independently colonised deep, clear waters in Lakes Malawi and Tanganyika.

The fate of cichlid radiations
Lakes are not necessarily closed environments over evolutionary timescales. Geological processes, such as tributary capture, mean that river catchments have fluid boundaries, and that fish can move between lakes and rivers. Lakes may also be sources of new forms that subsequently escape the catchment and spread more widely. For example, riverine *Lamprologus* in the Congo almost certainly arose

in Lake Tanganyika. Recently, it has been suggested that the diverse haplochromine cichlids of southern African river systems arose in a vast shallow lake in Botswana which has now dried out, forming the Makgadikadi salt pans. It has even been proposed that the haplochromine group as a whole arose in Lake Tanganyika, before spreading as far as Syria, Algeria and South Africa.

Cichlids have probably been diversifying in lakes for a long time: palaeontologists have found 45 million-year old fossils of five closely related cichlid species from what was a 400 m diameter crater-lake in Tanzania. So, adaptive radiations in lakes are not dead-ends, but are now thought to be the sources of evolutionary innovations that can lead to the colonisation of continental regions by new adaptive forms.

Conclusion
This article has focussed on the significance of cichlid fishes in the understanding of adaptive evolution and rapid speciation. As yet, there is no clear explanation for the propensity for some groups of cichlid fishes to undergo rapid speciation and diversification in African lakes. This is hardly surprising, as investigating such a question is likely to involve comparative study across many lineages in different localities, and most of these are found in areas in which local resources for research are comparatively poor. However, many significant issues have been raised and these have proved applicable to a wider community of researchers, such as the significance of hybridisation and sympatric speciation in animals. Genome sequencing could facilitate progress in these areas, for example through the identification of correlated rapid adaptive evolution and rapid genetic change in functional gene or regulatory element sequence, in gene expression levels or through structural rearrangements such as gene duplication. It may also help sort

out the phylogenetic relationships of closely related species and populations.

Of course, cichlids are interesting for other reasons apart from adaptive radiation. Most species live in species-rich but resource-poor areas of the world, particularly sub-Saharan Africa. Many species are believed to be threatened or possibly extinct, in particular the open-water species of Lake Victoria, where the ecosystem has changed drastically since the introduction of predatory Nile Perch. Tilapiine cichlids are among the most important freshwater food-fishes in tropical regions — they are palatable, tolerant of poor water conditions, fast-growing and easily bred. They are now cultured extensively outside their native range. Unlike many fishes cultured in temperate regions, they are largely herbivorous, making them ecologically and economically efficient to culture. On a crowded, warming planet, tilapia culture is likely play a significant role in human economy and food security, with artificial selection, hybridisation, preservation of wild genetic diversity and genetic modification all likely to be key issues. Thus, it is expected that sequencing of an African cichlid genome can only help stimulate substantial advances in evolutionary studies, conservation biology and aquaculture.

Further reading
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