How many species of cichlid fishes are there in African lakes?

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Abstract

The endemic cichlid fishes of Lakes Malawi, Tanganyika and Victoria are textbook examples of explosive speciation and adaptive radiation, and their study promises to yield important insights into these processes. Accurate estimates of species richness of lineages in these lakes, and elsewhere, will be a necessary prerequisite for a thorough comparative analysis of the intrinsic and extrinsic factors influencing rates of diversification. This review presents recent findings on the discoveries of new species and species flocks and critically appraises the relevant evidence on species richness from recent studies of polymorphism and assortative mating, generally using behavioural and molecular methods. Within the haplochromines, the most species-rich lineage, there are few reported cases of postzygotic isolation, and these are generally among allopatric taxa that are likely to have diverged a relatively long time in the past. However, many taxa, including many which occur sympatrically and do not interbreed in nature, produce viable, fertile hybrids. Prezygotic barriers are more important, and persist in laboratory conditions in which environmental factors have been controlled, indicating the primary importance of direct mate preferences. Studies to date indicate that estimates of alpha (within-site) diversity appear to be robust. Although within-species colour polymorphisms are common, these have been taken into account in previous estimates of species richness. However, overall estimates of species richness in Lakes Malawi and Victoria are heavily dependent on the assignation of species status to allopatric populations differing in male colour. Appropriate methods for testing the specific status of allopatric cichlid taxa are reviewed and preliminary results presented.

Keywords: African lakes, cichlid, evolution, species

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Introduction

The fishes of the family Cichlidae are a textbook example of 'adaptive radiation' or 'explosive speciation'. It is generally believed that there are more than a thousand species found in just three lakes in East Africa — Lakes Malawi, Tanganyika and Victoria. On the basis of current estimates of species richness, number of founding lineages and age of radiations, it appears that these fishes represent the most rapid known large-scale radiation of species of any animal group in a circumscribed area (Turner 1999a). There are many lineages of cichlid fishes and they differ in species richness, even when occurring in sympatry.

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Also individual lineages vary in species-richness between locations. Other, smaller lakes contain fewer species, and have been less intensively studied. Thus, we suggest that the study of cichlid fishes may offer unique opportunities to gain insight into the reasons underlying the taxonomic and geographical distributions of species richness and functional diversity.

Molecular phylogenetic studies have suggested that much of the radiation of species has occurred within the present basins of Lakes Malawi (Moran *et al*. 1994), Victoria (Meyer *et al*. 1990) and Tanganyika (Sturmbauer & Meyer 1993; Sturmbauer *et al*. 1994). Molecular studies have also provided circumstantial evidence for within-lake geographical isolation of populations confined to rocky shores of Lakes Tanganyika (Sturmbauer & Meyer 1992; Verheyen *et al*. 1996) and Malawi (van Oppen *et al*. 1997a; Arnegard

et al. 1999; Markert *et al.* 1999). This suggests a possible role for intralacustrine allopatric speciation among such habitat specialist taxa.

Recent theoretical studies have suggested that sympatric speciation is a more plausible process than previously believed, and that it might be driven by disruptive natural (Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999) or sexual (Turner & Burrows 1995; Higashi *et al.* 1999) selection, or by a combination of both (van Doorn *et al.* 1998). A study of several small lakes in Cameroon has provided strong evidence for sympatric speciation in endemic cichlids (Schliewen *et al.* 1994). Reviews of distributional evidence suggest that sympatric divergence may have occurred frequently in Lakes Malawi (Turner 1994) and Victoria (Seehausen & van Alphen 1999).

Divergence under natural selection, or at any rate co-existence in sympatry, may be facilitated by the legendary functional plasticity of the cichlid feeding structures (Galis & Drucker 1996), although it has been suggested that this 'key innovation' hypothesis requires further investigation (Turner 1999a).

However, not all groups of cichlid fishes are equally prone to explosive speciation (Turner 1999a) and even the lineages that are highly species-rich in clear-water lakes, are less diverse in habitats which, at least seasonally, have low water transparency (Seehausen *et al.* 1997). Studies of phylogeny, behaviour and ecology indicate that sexual selection by visually mediated female mate preferences has played a key role in the rapid speciation of many cichlid fishes, particularly the haplochromines which dominate Lakes Malawi and Victoria (e.g. Dominey 1984; McKaye 1991; Turner 1994, 1999a; Seehausen *et al.* 1997; Seehausen 2000).

Ultimately, however, the elucidation of factors responsible for the species richness of cichlid radiations must involve a rigorous comparative analysis of the relationships between the species richness of lineages and traits believed to be candidates for influencing the evolution of diversity. This kind of comparative study will depend critically on the use of phylogenetically corrected analyses, which means that reliable phylogenies will be needed (Pagel 1999; Dodd *et al.* 1999; Barraclough & Vogler 2000). Equally critical will be an accurate estimate for the numbers of species in particular lineages.

The purpose of the present review is to critically examine the evidence for these high estimates of species richness and to discuss methods by which the species status of cichlid taxa may be tested.

Species: concepts and practice

There is continuing debate over which species concept is most appropriate for the classification of fishes (e.g. Kullander 1999; Nelson 1999; Turner 1999b). Biologists studying living organisms tend to favour definitions of species that involve reproductive isolation (Mayr 1942) or at least a clustering of genetic variation (Mallett 1995). 'Biological' species status is a testable hypothesis for sexual taxa that are sympatric or parapatric with their sister taxon. Populations (in the statistical sense of groups of things you can collect data from, rather than necessarily the ecological sense of being geographically isolated portions of a species), which do not fit the biological species definition, can be assigned to the larger category of rather fuzzily defined 'genotypic cluster' species (Turner 1999b).

Many temperate lakes contain sympatric pairs of reproductively isolated fish species not formally recognized by taxonomists (Taylor 1999; Rundle *et al.* 2000). Until the 1980s, most cichlid species were defined on the basis of morphological traits likely to be amenable to study in preserved museum specimens (Turner 2000). Recent studies of cichlid fishes in Lakes Malawi and Victoria have emphasized the importance of male courtship colours in the definition of species (e.g. Ribbink *et al.* 1983; Seehausen 1996a; Turner 1996; Witte *et al.* 1997). Closer inspection of taxa defined on this basis has often led to the discovery of subtle differences in female colour, morphology, micro-habitat preferences or diet. A conspicuous exception to this must be made for taxa that are colour polymorphic and Seehausen *et al.* (1998a) suggest that as rule of thumb, sympatric species can be diagnosed by the correlated presence or absence of two or more (putatively) genetically independent characters. In this way, entities differing in colour, but otherwise apparently indistinguishable would be considered conspecific colour morphs.

What is a species flock?

The present review is principally concerned with the origin of cichlid species within a lake basin and will focus on 'species flocks' (Echelle & Kornfield 1984), which can be regarded as groups of two or more sister species endemic to a lake or lake basin. Lakes that contain only nonendemic species are obviously excluded. This definition would exclude cases such as the two endemic tilapiine cichlids of Lake Victoria, which are believed to be derived from two different lineages widespread within Africa (Trewavas 1983).

Discoveries of new species and species flocks

Several new species and even whole species flocks have been reported over the last decade. Konings (1995) listed approximately 575 Lake Malawi cichlid species, mostly from inshore habitats. Turner (1996) listed 199 species, 79 undescribed, from the deep-water and offshore habitats of Lake Malawi, 84 of which were not recorded by Konings, making a total of 659 between the two sources. Turner's

Table 1 African lakes possibly containing species flocks of endemic cichlid fishes

Lake	Number of cichlid species	Lineages with two or more endemic species	Reference
Malawi	≈700	Haplochromines, Tilapiines	Konings (1995); Turner (1996)
Victoria	≈700	Haplochromines	Seehausen (1996a)
Tanganyika	≈250	Various	Snoeks *et al.* (1994); Konings (1998)
Kyoga	≈100	Haplochromines	L. Kaufman, personal communication
Edward/George	≈60	Haplochromines	Snoeks *et al.* (1994)*
Kivu	18	Haplochromines	Snoeks (1994)
Albert	9	Haplochromines	Snoeks *et al.* (1994)*
Barombi Mbo	11	Tilapiines	Trewavas *et al.* (1972); Schliewen *et al.* (1994)
Bermin	8	Tilapiines	Stiassny *et al.* (1992); Schliewen *et al.* (1994)
Turkana	7	Haplochromines	Greenwood (1974); Snoeks (1994)
Rukwa†	5	Haplochromines	Seeghers (1996)
Nabugabo‡	5	Haplochromines	Greenwood (1965a)
Ejagham	4	Tilapiines	Schliewen *et al.* (1994)*
Natron/Magadi	4	Tilapiines	Seegers & Tichy (1999)

*These sources report estimates for species richness, but do not contain full species lists; †Rukwa may not contain a species flock, as it is possible that these species are derived from at least two lineages and that only two species actually live in the lake proper; ‡it is believed that each of the endemic species of Lake Nabugabo is independently derived from a different species of the Lake Victoria flock, and, if so, does not represent a true species flock.

study was conducted over a small part of the southern end of the lake, and subsequent work on a larger spatial scale has uncovered many additional species (J. Snoeks, personal communication). On the basis of the most conservative of species concepts employed by taxonomists currently actively working on the Lake Malawi cichlids, a minimum estimate for the total cichlid species count must be in the region of 700.

Seehausen (1996a) recorded 173 cichlid species from the rocky habitats of Lake Victoria, 100 of which were discovered since 1986. Again, most of this work was undertaken in a small area of the lake, and many of the species identified were found to be resticted to parts of the surveyed area, suggesting that more remain to be discovered. Recent preliminary surveys of rocky shores in Ugandan parts of the lake also indicate restricted distributions of species (O. Seehausen, S.B. Wandera and L. Kaufman, unpublished data). In addition, more than 200 species were recorded from nonrocky habitats of the lake prior to their extermination during the Nile Perch population explosion (Witte *et al.* 1992). Seehausen (1996a) reported the total count of currently known cichlid species for the pristine Lake Victoria at over 500, and estimated the number of as yet unknown species at over 200, resulting in a total estimate of about 700 or more species.

Recent surveys of Lake Tanganyika have also led to increasing estimates of cichlid species richness in that lake from 173 to more than 200 (Snoeks *et al.* 1994). A recent field guide listed 213 species (Konings 1998) and 250 might be a good rough estimate of the total likely to be found eventually.

New species, and indeed species flocks, continue to be reported from smaller water bodies (Table 1). These new taxa are not the results of a taxonomic splitting frenzy, but rather the majority of these forms not previously having been studied or collected.

As part of the comparative approach we advocate, it may also be instructive to study lakes in which cichlids have not undergone speciation. In some cases, this is likely to be due to the absence of cichlids from the catchment or to the recency of origin of the lake. However, Lake Tana in Ethiopia has had time to evolve an endemic flock of perhaps 13 species of cyprinid fishes of the genus *Barbus* (Nagelkerke & Sibbing 1996), but also contains a population of the widely distributed tilapiine cichlid *Oreochromis niloticus* (Trewavas 1983). Another intriguing case is that of *Tilapia guinasana*, which is endemic to a single sinkhole in Namibia, which measures only 70 × 40 m (Ribbink *et al.* 1991). Although over 200 m deep, fish generally live only in the upper 20 m, near the sheer rock walls. The species is found in 3–6 colour forms. Mating is only weakly assortative — 1.4 times more homomorphic pairs than expected by chance (reanalysed from Ribbink *et al.* 1991) — but random amplified polymorphic DNAs (RAPDs) indicated considerable genetic differentiation among the morphs (Nxomani *et al.* 1999).

Morphospecies, resource polymorphism and phenotypic plasticity

Many species have been formally described from preserved specimens, on the basis of diagnostic features associated with the collection and processing of food (e.g. Eccles &

Trewavas 1989). Could variation in such features be the result of intraspecific variation? If so, the total species counts might be greatly overestimated.

Many studies of temperate lake fishes have suggested that sympatric forms often differ mainly in traits associated with feeding and habitat use (Skúlason *et al*. 1999; Taylor 1999). It is not clear whether these 'resource polymorphisms' arise through disruptive selection within a single gene pool, or whether they result from contact between populations previously split by geographical barriers or by intrinsic reproductive isolating traits.

Phenotypic plasticity

Phenotype plasticity of cichlid feeding structures has been documented for *Astatoreochromis allaudi* from the Lake Victoria region (Greenwood 1965b; Hoogerhoud 1986), for other Lake Victoria species (Witte *et al*. 1997) and various other African and Neotropical cichlid species (Meyer 1987, 1990; Kornfield 1991; Wimberger 1991). These studies have generally involved splitting a single clutch of young and rearing them on different diets. Changes have been observed in the shape of teeth, oral and pharyngeal jaws, and the head in general. These differences are sometimes of the nature and magnitude that have been used to distinguish subspecies, species or even genera of cichlids.

Geographic variation

Geographic variation in feeding structures is known in several species, such as *A. allaudi* in the Lake Victoria catchment (Greenwood 1965b; Hoogerhoud 1986), and the Malawian *Lethrinops mylodon* (Eccles & Trewavas 1989) and *Oreochromis karongae* (Turner & Robinson 1991). The differences between these geographical variants are sometimes as substantial as among species or indeed genera (Hoogerhoud 1986; Witte *et al*. 1997) recorded from within the same lake. If, as is often the case, species are known only from a few specimens from unknown locations within the lake, they might simply represent conspecific allopatric variants. In most studies, it is unclear if such variation has a genetic basis. Bouton *et al*. (1999) found that in three species of rock-dwelling Lake Victoria cichlids (*Neochromis*), the development of the bones of the jaws varied among locations according to the availability of different food items. In populations where more of their food required biting, the fish were capable of delivering a stronger biting force. Anecdotally, they reported that, for one species at least, the anatomical differences between two populations remained after two generations of breeding in the laboratory under standardized conditions, implying a genetic basis for geographical variation. If verified and found to be generally applicable, this would be a highly significant finding, suggesting rapid adaptive evolution of anatomical traits

associated with feeding in these fishes. If there is a genetic basis to such differences, allocation of species or subspecies status to some of the more strikingly different variants might be justified, if a morphospecies concept were adopted.

Intrapopulation polymorphism

Intrapopulation polymorphism is clearly shown in the case of the left- and right-handed morphs of the Lake Tanganyika scale-eating cichlid fish *Perissodus microlepis* (Hori 1993). Although indicative of a possible frequency dependent selective mechanism for the maintenance of polymorphism, these forms have never been regarded as different species. However, in several Central American cichlid species, there is clear evidence for resource polymorphisms of the nature and magnitude used by morphological taxonomists to distinguish species. For example one morph of *Cichlasoma minckleyi*, exhibits enlarged pharyngeal bones with molariform teeth for crushing molluscs, and other has more normal 'papilliform' dentition. This polymorphism has been shown to have a genetic basis, as both morphs were found in crosses, even when reared on a common diet of soft foods (Kornfield 1991). Similar discrete polymorphisms have been reported in Malawian endemics, including *Labidochromis caeruleus* (Lewis 1982) and *Mylochromis semipalatus* (Konings 1995). Irrespective of the genetic bases of these traits, these results suggest that some species defined on such morphological criteria may be merely conspecific trophic morphs.

Cryptic species

Cryptic species may have been detected by a recent mitochondrial DNA (mtDNA) phylogeny of the Lake Tanganyikan eretmodine cichlids by Rüber *et al*. (1999). This phylogeny bears little relationship to the current taxonomy, which is based largely on tooth form. Rüber *et al*. suggest that similar trophic morphologies have arisen on a number of occasions, perhaps in response to similar ecological selection pressures, and thus, that the number of species in this lineage has been underestimated. However, there have been several other studies of African cichlid fishes where gene trees and morphological species trees have disagreed. Rather than reject morphological classification, the authors of most of these studies have suggested that the mismatch is likely to be due to incomplete lineage sorting of molecular markers (Klein *et al*. 1993; Moran & Kornfield 1993; Parker & Kornfield 1997; Sato *et al*. 1997). This is possibly a result of the short interval between speciation events and the consistently large population sizes of most species (Nagl *et al*. 1998). So, are Rüber *et al*. justified in rejecting morphological taxonomy, and arguing for a higher species count? The existing eretmodine taxonomy is based on characters likely to be phenotypically plastic

and prone to homoplasy. Their phylogeny does not look like it has a high ratio of phylogenetic noise to signal. There is a clear phylogeographic pattern. Two ancient lake-wide lineages have each been subdivided into clades conforming approximately to the outlines of the three ancient basins which were split apart by documented falls in the water level of the lake. However, mtDNA studies on Hawaiian crickets have shown a similar phylogeographic pattern which disagrees with morphological classification (Shaw 1996), but largely nuclear AFLP analysis has indicated that this could be due to repeated introgression (K.L. Shaw, personal communication). Introgression could cause incomplete lineage sorting. The picture is still unclear.

In conclusion various studies have demonstrated that apparent resource polymorphism may be due to developmental plasticity, while others have hinted at possible cases of genetically based polymorphisms. Both of these lines of evidence would tend to support the view that the number of cichlid species might be overestimated. However, the study by Bouton *et al*. (1999) suggests a potential for rapid genetic evolution of such traits, and the work by Rüber *et al*. (1999) suggests that there may be parallel evolution of such traits, and thus, that species numbers are underestimated in some cases. There are no published studies of the relationship between reproductive isolation and differentiation of feeding structures among taxa that do not differ in courtship traits, such as breeding colour. Thus, it is difficult to relate any of these findings to the biological species status of the taxa in question. It is our guess that whatever general pattern eventually emerges, the effect on estimates of species richness for African cichlid flocks is unlikely to be influenced by more than ±5%.

Colour polymorphism

Recently, taxonomists working on African cichlids have granted species status to forms that differ strikingly in colouration, but in little else (reviewed in Turner 2000). On the other hand, some cichlid species are apparently colour polymorphic. How are these factors presently reconciled, and how does this influence the estimates of species richness?

Largely female-limited colour morphs

The most striking manifestations of colour polymorphisms are the occurrence of blotched or otherwise conspicuously coloured females in species where the majority of females are drab brownish or grey. Very similar barred/blotched/orange polymorphisms have been identified among the endemic haplochromine cichlids of Lakes Malawi (Konings 1995), Victoria (Seehausen 1996a) and Kivu (Snoeks *et al*. 1989). Some populations of the Malawian *Pseudotropheus callainos* have three female morphs, all of them conspicuous to the human eye — blue, white and white-blotched. Other

blotched forms are phenotypically very different. Within Lake Tanganyika, orange-blotched females are common in the haplochromine *Ctenochromis benthicola* (Konings 1998). Blotched individuals of both sexes are occasionally found in tropheine (Konings 1998) and eretmodine (Wheeler 1999) species from Lake Tanganyika, and in the Lake Victoria tilapiine *Oreochromis variabilis* (Trewavas 1983; Seehausen 1996a).

The most thoroughly studied case is that of *Neochromis omnicaeruleus* from Lake Victoria, where seven male and 10 female colour morphs have been recorded in sympatry (Seehausen *et al*. 1999a). The commonest female forms are: (i) brown with dark vertical bars (P); (ii) white with black blotches (WB); and (iii) orange with black blotches (OB). To simplify greatly, the WB- and OB-determining genes seem to be at different loci. The common blotched forms appear to be heterozygotes. Homozygotes are either almost entirely orange (OB) or almost black (WB). In nature, 99% of the males are plain blue, which is the equivalent of the P female, but occasional OB and WB males occur. Seehausen *et al*. (1999a) found that the frequencies of the different morphs produced in laboratory crosses are consistent with a rather complex model, which, involves tight linkage between the blotched pattern (both WB and OB) and a dominant female-determining W gene which can lead to sex-ratio distortion. Autosomal 'male rescue' genes, which are present in some individuals, override the female determining effect of the W-gene, producing blotched males, while having the effect of enhancing the blotched pattern in females.

The function of female polychromatism in cichlids is mysterious. It is striking that in most cases, this polymorphism is confined to polygynous maternal mouthbrooding species, and is more common in females than in males. This recurrent pattern suggests that selection pressures on colour differ between the sexes (Seehausen *et al*. 1999a). A similar pattern is found in some *Papilio* butterflies, where different female morphs mimic different distasteful 'models'. In the butterflies, it is male monomorphism which is the puzzle, and it has been suggested that this may be due to female preferences, male–male competition or higher predation pressure on females (reviewed by Joron & Mallet 1998). In cichlids, laboratory experiments have shown that females of the plain morph of *N. omnicaeruleus* do indeed prefer plain morph males, but the blotched morph females court at random with respect to male colour (Seehausen *et al*. 1999a). Preliminary observations with the polymorphic Malawian *Pseudotropheus zebra* also show that some females mate with blotched males, even when a 'normal' barred male is present.

Frequencies of *P. zebra* colour morphs differ significantly between the north and south sides of a 700-m wide bay at Nkhata Bay, Malawi (Knight 1999). With restricted gene flow across such habitat barriers (van Oppen *et al*. 1997b),

geographical variation in frequencies of colour morphs could be selectively neutral. However, in the same study, frequencies of colour morphs were also found to vary between depths. For both headlands, the trend was similar, with higher densities of the bright orange (O) morph *P. zebra* in shallower water. Variation in frequencies over such a narrow depth gradient calls for a selective explanation, but it is not clear what it might be. The higher abundance of O females in shallow water is the reverse of what would be expected if predation on bright fish was the dominant selective force. The O morph females are extremely conspicuous to human eyes, and might be expected to suffer higher predation from visual hunters with colour vision, such as cormorants and kingfishers. In deeper water, the principal predators are likely to be noctural nonvisual predators such as the tactile/olfactory catfishes and electrosensory mormyrids.

Could female polymorphism be maintained by male preference? Male *N. omnicaeruleus* seem to be polymorphic for mate preference, with offspring of plain × plain matings preferring plain females and offspring of blotched × blotched matings preferring blotched females (Seehausen *et al*. 1999a). It appears that individual male *P. zebra* may also express morph-based preferences.

In Lake Malawi, there are no known cases of this kind of polymorphism in haplochromines from the more turbid sandy and muddy habitats. Likewise, in Lake Victoria, Seehausen *et al*. (1999a) observed that blotched females are rarer in areas of lower water transparency. This pattern repeats itself on a larger geographical and taxonomic scale. Blotched and orange female morphs are apparently largely confined to lacustrine cichlid species (Kornfield 1991; Witte *et al*. 1997). It seems that blotched and orange females tend to occur in places where they can be seen, and so it seems unlikely that their occurrence could be selectively neutral, or due to pleiotropy. Irrespective of the possible function, it has been suggested that this kind of polymorphism could be a precursor to speciation based on mate preferences (Seehausen *et al*. 1999a).

Non sex-limited

There seems to be a clearer functional explanation for a different kind of colour polymorphism in Lake Tanganyika. Within a single site, Kohda & Hori (1993) found that eight of 19 carnivorous cichlid species exhibited light/dark polychromatism. All were active diurnal predators of evasive prey, such as fishes and shrimps. A more detailed study of one of these species, *Lepidiolamprologus profundicola* revealed that it employed nine different behavioural strategies in capturing its fish prey. Most individuals have one or two preferred strategies and these preferences were found to be stable over observational periods of 1–5 months. Dark morph individuals tended to specialize in attacking from on or under rocks, while pale morph individuals generally attacked from over the sand or from the water column (Kohda 1994). We are unaware of any reports of investigations of the genetic basis of either the behavioural or colour polymorphisms of these species. The differences could be due to phenotypic plasticity, and may even be reversible.

Male-limited colour polymorphisms

A third category of colour polymorphism affects male nuptial colouration. Two major types can be distinguished among Lake Victoria cichlids. The less common and less conspicuous one is fin colour polymorphism, where males with bright red and bright yellow anal fins, or red vs. blue caudal fins occur within a population. The more common one is body colour polymorphism, where bluish males co-occur with yellowish males with a red chest and/or yellowish males with a red dorsum. This latter type of polymorphism has been found in 18 species of rock-dwelling cichlids in Lake Victoria and is known from many nonrock cichlids too (Seehausen *et al*. 1999b). The differences between conspecific morphs strikingly resemble differences between closely related sympatric species in Lake Victoria, which has led to the hypothesis that disruptive sexual selection operating on these polymorphisms could result in speciation.

Within Lake Malawi, some populations of *Cynotilapia afra* on Likoma Island exhibit a yellow/white dorsal fin polymorphism (Ribbink *et al*. 1983). We have also found this kind of polymorphism in the same species at Nkhata Bay on the opposite side of the lake. The possibility that these morphs represent sympatric species has yet to be tested.

In general, largely female-limited and nonsex-limited colour polymorphisms within species have already been recognized as such by taxonomists and accommodated into more recent estimates for species richness.

Species status

Tests of sympatric taxa in the field

The specific status of sympatrically occurring taxa has been tested by behavioural observations of assortative mating, by molecular analysis of gene frequencies at several unlinked loci and by controlled breeding in the laboratory.

Observations of assortative courtship and other ecological differences among species of the Malawian *Pseudotropheus zebra* complex (Holzberg 1978; van Oppen *et al*. 1998), the *Pseudotropheus tropheops* complex (Ribbink *et al*. 1983; van Oppen *et al*. 1998) and the genus *Petrotilapia* (Marsh *et al*. 1981) have been supported by genetic differences assayed by allozymes (McKaye *et al*. 1982, 1984) and microsatellites (van Oppen *et al*. 1998).

Similar molecular studies have not yet been carried out with cichlid fishes from other lakes. However, behavioural

studies by Seehausen *et al.* (1998b) demonstrated that assortative courtship among eight putative species of sympatric rocky-shore Lake Victoria cichlids occurred significantly more often than would be expected on the basis of their relative frequencies. Only 41% of encounters, but 79% of lateral displays, 96% of quivers and 100% of leads and circles were with conspecifics. These activities are initiated at successively later stages in the typical courtship sequence. Thus, species recognition improved as the courtship sequenced progressed further towards spawning. In controlled breeding experiments, two sympatric species of *Pundamilia* and three of *Neochromis* that differed in colour and also in other morphological traits were found to be true breeding.

Estimates for alpha diversity

Molecular and behavioural studies have thus generally provided support for previous high estimates of alpha diversity in Lakes Malawi and Victoria.

On the rocky shores of Lake Malawi, 12–44 species of the 'mbuna' complex are reported to co-occur within a habitat patch (Ribbink *et al.* 1983; Reinthal 1993), but mbuna are not the only cichlids found in these habitats. A rare estimate of total cichlid diversity was made by Robinson (1995), who recorded 64 species along a 700-m transect covering depths of 0–12 m at Thumbi East Island. Strikingly, the species richness of the mbuna at this location was among the lowest recorded, at 15. Alpha diversity is also estimated to be high in the nonrocky habitats in Lake Malawi. In a trawl survey in the south-eastern arm of Lake Malawi from February to May 1992, 67 hauls of 20 min duration were carried out at 34 sites at depths of 18–128 m. The number of haplochromine cichlid species per haul varied from nine to 39 (G.F. Turner, unpublished data).

Between seven and 23 species of rock-restricted cichlids (up to 18 species of the 'mbipi' complex) have been found at single rocky island in Lake Victoria (Seehausen & Bouton 1998). The number is strongly positively correlated with water transparency (Seehausen *et al.* 1997). The maximum number of nonrock-restricted species that can be encountered in a single rocky patch is currently about 10, most species coming from adjacent sandy beaches. This number was higher before the Nile Perch boom that removed several trophic groups and most open water species from the system. The maximum observed total species number at a rocky island is 28 at Makobe Island, although it has few nonrock cichlids due to its isolation. Alpha diversity in nonrocky habitats had been very high before the Nile Perch boom. In 1979/80 28 bottom trawls of 10 minutes, over 13–15 m depth on a research transect, yielded 42 different species over a mud bottom (Witte *et al.* 1999), and a similar number of trawls in 2–6 m depth yielded about 60 species (Witte *et al.* 1992).

Table 2. The relationship between estimated total and alpha species diversities of cichlid communities in the African Great Lakes (sources in text)

	(a) Maximum reported alpha diversity	(b) Estimated total species number	(b/a)
Lake Malawi	64	700	10.9
Lake Victoria	60	700	11.7
Lake Tanganyika	45	250	5.6

Hori (1997) reported 41–47 species, mostly cichlids (only two noncichlid fishes appear on the accompanying food-web diagrams), from a series of 400 m² quadrats on the rocky shores of Lake Tanganyika.

The depths of Lakes Malawi and Tanganyika are deoxygenated and are believed to contain no fish (Coulter 1991; Menz 1995). Otherwise, the lowest levels of alpha diversity have been reported from the pelagic waters over the anoxic zones. Cichlids are absent from the true pelagic in Lake Tanganyika (Coulter 1991). On Lake Malawi, we recently participated in a pelagic trawl survey comprising 20 hauls taken throughout the surface waters of the lake. Although approximately 20 000 fish, totalling 1044 kg were examined, only 11 cichlid species were collected, and of these only five species were common (data reanalysed from Turner *et al.* 2000).

The problem of allopatric taxa

The high estimates of species richness in Lakes Malawi and Victoria depend critically, not only on the alpha diversity, but also on the level of beta (between site) diversity. Clearly, the previously noted low levels of species richness on Lake Tanganyika (Turner 1999a) are strongly influenced by that lake's much lower apparent level of beta diversity (Table 2). Many Tanganyikan taxa presently regarded as single species show enormous levels of geographical variation, especially in colour (e.g. Konings 1998: pp. 40–41).

Taxonomists, even those working on the same lake, differ in their willingness to assign species status to allopatric taxa, and also seem to differ in the practices they adopt with different groups of species. Ribbink *et al.* (1983) stated that they maintained conspecific status for taxa that could be consistently matched up with allopatric forms, even when they differed markedly in colour. Thus, the genus *Labeotropheus* was considered to comprise two species, despite extensive geographical variation in colour. This was because they recorded no more than two sympatric forms, and in such cases, one was always a deep-bodied form mainly found in shallow water (*L. fuelleborni*) and the other a long-bodied form in slightly deeper water

Table 3 Estimates for levels of total species diversity in Lake Malawi are strongly dependent on assignations of species status to allopatric forms (data reanalysed from Ribbink *et al*. 1983)

Species complex	Number of species	Maximum number of sympatric species
Pseudotropheus zebra	27	6
P. tropheops	34	7
P. williamsi	8	1
P. elongatus	24	3
P. 'aggressive'	12	4
P. 'miscellaneous'	20	4
Melanochromis A	15	5
Melanochromis B	5	3
Petrotilapia	17	4
Labidochromis	17	4
Cynotilapia	10	4
Gephyrochromis	2	1
Labeotropheus	2	2
Iodotropheus	1	1
Cyathochromis	1	1
Genyochromis	1	1
Total	196	51

(*L. trewavasae*). However, the same authors recognized eight species of the *Pseudotropheus williamsi* complex, although they never recorded more than a single form at any site. We have reanalysed the distribution data of Ribbink *et al*. (1983) for the mbuna group. A rough minimum estimate for the total number of species can be given as the sum of the maximum number of sympatric species recorded for each of the 16 species complexes they recognized. This gives a figure of 51 species, compared to Ribbink *et al*.'s estimate of 196 (Table 3). The estimate of 51 is probably overly conservative, as we have not checked for cases where three species never co-occur, but all possible sympatric pairs of two taxa can be found at different sites, indicating that all three are biological species. Still, this meta-analysis indicates that the high estimates for total species richness of Lake Malawi are strongly dependent on assumptions of the specific status of allopatric forms.

Tests of species status in the laboratory

Tests of species status of allopatric forms can be carried out only in the laboratory, as it would be unethical to carry out deliberate transplantation of populations to areas of the lakes where they were not indigenous. Laboratory studies have long been used to test for reproductive isolation in other groups of organisms, although there are important methodological considerations to be borne in mind (e.g. for *Drosophila*: Coyne & Orr 1997; Casares *et al*. 1998; for sticklebacks: Rundle *et al*. 2000).

1. No choice (NC) experiments

Many species of cichlid fishes will hybridize in laboratory conditions, in absence of opportunities to select conspecific mates (Crapon de Caprona & Fritzsch 1984; McElroy & Kornfield 1993; Seehausen *et al*. 1997; Knight *et al*. 1998). Some evidence of postmating barriers has been found in haplochromines. For example, a lethal cross has been found between female *Astatotilapia nubila* (Lake Victoria catchment) and *A. burtoni* (Lake Tanganyika catchment), although the reciprocal cross is viable and fertile to F2 at least (Crapon de Caprona & Fritzsch 1984). In many cases F1 and F2 hybrid offspring are viable and fertile, even in crosses between species from different genera, although sex ratios may be highly skewed (Crapon de Caprona & Fritzsch 1984; McElroy & Kornfield 1993; Seehausen *et al*. 1997). We have produced intergeneric hybrids of Lake Victoria cichlids (*Pundamilia* × *Platytaeniodus*) that have not shown any evidence of loss of viability or fertility up to the 5th generation. Sympatric species that do not interbreed in nature will sometimes hybridize under 'no choice' (NC) conditions in the laboratory (Seehausen *et al*. 1997; Knight *et al*. 1998). Although Crapon de Caprona & Fritzsch (1984) used time until spawning in a NC set up as a measure of mate preferences, we have found that this is very dependent on the age and health of the fish and the feeding regime. Thus, NC experiments would appear to offer little insight into species status of allopatric taxa.

2. Free contact (FC) experiments

In 'free contact' (FC) experiments, males and females of two or more test populations are allowed to mix freely in the experimental tanks. Mate preferences can be scored by direct observation or by molecular paternity testing of the offspring.

Three species of the *P. zebra* complex co-occur at Nkhata Bay, in Lake Malawi: *P. zebra*, *P. callainos* and the undescribed *P.* 'gold zebra'. Laboratory studies using microsatellite DNA to test paternity indicated that 264 offspring from 22 broods produced in three replicate tanks were all the result of matings between conspecifics (Knight *et al*. 1998). These species are known to represent independent gene pools in the natural habitat (van Oppen *et al*. 1998), but the laboratory study permits us to conclude that assortative mating is the result of direct mate preferences, rather than differences in reproductive season or breeding microhabitat choice. Seehausen (1996b) assayed direct observations of spawning using the FC design to demonstrate complete assortative mating among a pair of sympatric species of the genus *Pundamilia* from Lake Victoria.

The FC design has since been used to test for assortative mating among allopatric taxa. On the north-western shore of Lake Malawi, *P. zebra* and *P. callainos* both occur as a

number of allopatrically distributed colour forms that are considered by some authors to represent species (Ribbink *et al.* 1983; Stauffer *et al.* 1997). Two such races from both of these species have been tested following the methods of Knight *et al.* (1998). Not all broods have been typed but preliminary results clearly indicate that individuals of the two populations of *P. zebra* have hybridized, but that this is not the case for *P. callainos* where so far complete assortative mating between the two races has been observed. It is notable that the test populations of *P. callainos*, unlike those of *P. zebra*, differed in female as well as male colour. We have shown that males of the Nkhata Bay population of this species could distinguish conspecific females (of all morphs) from those of sympatric heterospecifics on the basis of colour alone (Knight & Turner 1999). Thus, the complete assortative mating (if confirmed) among populations of this species could be due to male as well as female mate choice.

However, it is possible that the production of hybrids in this experiment could be due to an overriding of female choice by dominance relationships among the *P. zebra* males.

3. Closed chamber (CC) experiments

In aquaria, females may not be free to exercise choice among males as limited space, and individual differences in competitive ability may lead to a situation where only males of a single species (or form) can establish and defend mating territories. Thus, it is fundamental to the FC design that we can only draw positive conclusions about species status from observations of complete assortative mating, such as those of Knight *et al.* (1998). In a study of mate preferences of the sympatric Lake Victoria cichlids now known as *Pundamilia nyererei* and *P. pundamilia* (Seehausen *et al.* 1998a), Seehausen & van Alphen (1998) confined males in tranparent perspex enclosures. Female mate preferences were scored as the proportion of male displays that were followed by approaches by the females. This study demonstrated clear preferences by females for conspecific males under white light, but under orange artificial light, females do not distinguish among the males of the two forms. This experiment mimicked the situation in Lake Victoria. Around islands where there is high water transparency, the blue and red forms are clearly distinct and are believed to represent reproductively isolated species. In other places the water is turbid, and the transmitted light spectrum narrowed (Seehausen *et al.* 1997). In such habitats, the red forms are more drab, and intermediate forms more frequent, perhaps as a result of introgression (Seehausen 1996b; Seehausen *et al.* 1997). These studies suggest that the genetic integrity of a species is dependent on environmental parameters, and perhaps even that forms that are different species in some places, are conspecific morphs elsewhere.

The CC method was also employed in the study by Seehausen *et al.* (1999a) of mate preferences among conspecific morphs of *Neochromis omnicaeruleus*, and in Knight & Turner's (1999) test of preferences of males of the *P. zebra* complex for conspecific females.

Although the CC method avoids the risks of male–male competition masking mate preferences, it is possible that behavioural response measures may not always be an adequate predictor of actual spawning decisions. Female mouthbrooding cichlids often follow and court with many males, but spawn with only a few of them (e.g. Taylor *et al.* 1998).

We are currently running an FC experiment with colour morphs of *N. omnicaeruleus* to test whether the male mating preferences that were observed in CC experiments translate into mating patterns in the FC design. To do so, we have one blue and one blotched male, and five plain brown and five blotched females free ranging in a 3-m long tank. Behavioural courtship observations have confirmed the mating preferences observed in the CC design, but as in a previous study (Seehausen 1996b), they were even more distinct in the FC situation, where the blue male courted blotched females and the reverse. Preliminary results using amplified fragment length polymorphism (AFLP) to analyse paternity of clutches obtained from three blotched and five plain females confirm the visually observed mating preferences (E. Koetsier, unpublished data). Hence, in both studies that used CC and FC, the two methods gave consistent estimates of the direction of preferences, but the CC method tended to underestimate the strength of preferences.

The advantage of the CC method is that it has the potential to permit the testing of the repeatability of a female's preference during a single breeding cycle, by allowing her to choose among several different pairs of males. Thus, it can potentially be used to generate a large data set in a comparatively short period of time.

4. The partial partition method (PP)

The 'partial partition method' (PP) has both the advantage of the CC method in avoiding male–male competitive interactions, and the benefit of the FC method in allowing full course of mating to be completed. Hert (1989, 1991) employed this method to study sexual selection in *Haplochromis squamipinnis* from Lake George and *Pseudotropheus aurora* from Lake Malawi. Hert confined the larger males by the use of mesh partitions that permitted the smaller females to move freely within the tank and spawn with their chosen males. Preferences were determined by direct observations of spawning. The study demonstrated that females preferred to mate with conspecific males that had greater numbers of yellow 'egg-dummies' on their anal fin.

We are employing the PP design to test for assortative mating among five geographical races of *P. zebra*. Although the experiment has not yet been completed, preliminary microsatellite analysis has already demonstrated one case of hybridization. As the males of these populations differ in several aspects of their breeding dress, perhaps these traits are not important in mate selection by females. Alternatively females may be 'bet-hedging' to maximize the genetic variability of their offspring by mating with several males, as has been shown to occur in the field in this and other Lake Malawi cichlid species (Kellogg *et al.* 1995; Parker & Kornfield 1996).

Conclusions

Estimates of alpha diversity indicate that there are a great many cichlid species in Lakes Malawi, Tanganyika and Victoria. However, the numbers of species in each lake, and in particular lineages within lakes, are still poorly known. Measures of both alpha and beta diversity generally vary according to sampling effort, and the studies we cite above vary greatly in effort, sampling methods and skill of practitioners in the identification of species.

Molecular methods have provided the tools required to distinguish between sympatric species and morphs, on the basis of field samples. However, this does not solve the problem of allopatric taxa, which contribute significantly to the total estimates of species numbers, especially in Lakes Malawi and Victoria. Consequently, it is not yet clear whether Lake Tanganyika really has far fewer cichlid species than Lakes Malawi and Victoria, as has been claimed (e.g. Turner 1999a).

The determination of the specific status of allopatric forms is likely to rely on laboratory testing of assortative mating, and the best available method appears to be the partial partition method. However, the closed chamber method has the advantage of being able to generate more data in a shorter period of time.

If taxa are fully reproductively isolated in the laboratory, then they are very likely to be good biological species. However, an important criticism of laboratory mating studies is that if allopatric taxa interbreed in the laboratory, it is possible that they would not do so in the field. Interbreeding of allopatric species may be due to two factors: direct mate choice may be constrained by laboratory artefacts or reproductive isolation may depend critically on environmental heterogeneities that do not exist in the controlled condition of the laboratory. In nature, there may be hundreds of courting males for a female to choose from. In cichlids, where males remain in their territories during the mating period, females are free to wander around and sample many males. In small aquaria, a single dominant male may prevent other males, including those of other species, from establishing territories and courting females. We believe that the PP and CC designs largely or entirely circumvent this problem. Correct lighting conditions must be provided, as it is known that a limitation of spectral bandwidth may lead to a loss of assortative mating among species (Seehausen *et al.* 1997). Account should be taken of the recent finding that some cichlid species can see ultraviolet light (Carleton *et al.* 2000). However, these problems are not insurmountable. Furthermore, the elimination of spatio-temporal heterogeneity in the laboratory is only a problem if species are reproductively isolated by these factors in nature. Our studies suggest that in many pairs of sympatric cichlid species, reproductive isolation may be entirely due to direct mate preferences. Perhaps this is a general, or even universal, pattern among cichlids.

These laboratory studies have the potential to reveal which traits are actually important in the maintenance of reproductive isolation between species. To do so has two important consequences. First, it may help to distinguish among factors that may be candidates for influencing speciation, such as female choice, male choice, ecological differentiation, and postmating barriers. Second, to clarify the traits responsible for reproductive isolation will facilitate the development of useful 'rules of thumb' for the allocation of specific status to taxa which do not occur sympatrically with putative sister taxa. After all, while it will be interesting to determine the first few cases of assortative or nonassortative mating among allopatric taxa differing in male colour, to do so for all African lake cichlids would be extraordinarily expensive and tedious.

A major gap lies in the investigation of reproductive isolation among taxa that differ primarily in trophic morphology rather than in colour. It is known that endemic cichlids from Lake Tanganyika (Nelissen 1991) and Lake Malawi (Lobel 1998) produce sounds during courtship. It is possible that different developments of pharyngeal jaw structures may affect sound production and this could lead to assortative mating on the basis of internal morphology. It is also known that male haplochromine cichlid fishes respond to the olfactory cues emitted by gravid conspecific females, although discrimination has only been shown among distantly related taxa from different continents (Crapon de Caprona 1982). Such sensory modalities may be more significant in nonhaplochromine species, and in these species, there are few studies of assortative mating.

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