

Evolution of colour patterns in East African cichlid fish

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Abstract

African cichlid fishes have undergone outbursts of explosive speciation in several lakes, accompanied by rapid radiations in coloration and ecology. Little is known about the evolutionary forces that triggered these events but a hypothesis, published by Wallace Dominey in 1984, has figured prominently. It states that the evolution of colour patterns is driven by sexual selection and that these colour patterns are important in interspecific mate choice, a combination which holds the potential for rapid speciation. Here we present phylogenetic analyses that describe major events in colour evolution and test predictions yielded by Dominey's hypothesis. We assembled information on stripe patterns and the presence or absence of nuptial coloration from more than 700 cichlid species representing more than 90 taxa for which molecular phylogenetic hypotheses were available. We show that sexual selection is most likely the selection force that made male nuptial coloration arise and evolve quickly. In contrast, stripe patterns, though phylogenetically not conserved either, are constrained ecologically. The evolution of vertical bar patterns is associated with structurally complex habitats, such as rocky substrates or vegetation. The evolution of a horizontal stripe is associated with a piscivorous feeding mode. Horizontal stripes are also associated with shoaling behaviour. Strength of sexual selection, measured in terms of the mating system (weak in monogamous, strong in promiscuous species), has no detectable effects on stripe pattern evolution. In promiscuous species the frequency of difference between sister species in nuptial hue is higher than in pair bonding and harem forming species, but the frequency of difference in stripe pattern is lower. We argue that differences between the two components of coloration in their exposure to natural selection explain their very different evolutionary behaviour. Finally, we suggest that habitat-mediated selection upon chromomotor flexibility, a special form of phenotypic plasticity found in the river-dwelling outgroups of the lake-dwelling cichlids, explains the rapid and recurrent ecology-associated radiation of stripe patterns in lake environments, a new hypothesis that yields experimentally testable predictions.

Introduction

With between 2000 and 2500 known species, cichlids (Teleostei, Cichlidae) are probably the most species rich

family of vertebrate animals. With over 1500 species, more than half of the world's cichlid species live in the African Great Lakes, where several endemic species flocks evolved in parallel. Speciation rates in some of these flocks are unrivaled among vertebrates and they are among the most colourful animal species assemblages on Earth. Recent studies have attempted to link the ecological and morphological diversity of cichlids with their diversity in male nuptial coloration (McElroy *et al.*, 1991; Deutsch, 1997; Seehausen *et al.*, 1997). Nuptial coloration, however, is but one aspect of cichlid colour

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diversity. We address here the evolution of the major colour traits in East African cichlids; the stripe patterns formed by melanophores, and the origin of nuptial coloration, formed by structural and carotenoid colours.

Coloration in fish has diverse functions and is often under strong natural selection (Endler, 1980, 1983, 1986). Fish have many visual predators (Endler, 1978, 1991; Milinski, 1993) and themselves prey upon visually well-equipped organisms such as arthropods and other fish (Guthrie & Muntz, 1993). Coloration in fish is often important in intraspecific communication (Guthrie & Muntz, 1993), can be under intraspecific sexual selection by female choice (Kodric-Brown, 1985; Houde & Endler, 1990; Milinski & Bakker, 1990), by male–male competition (Evans & Norris, 1996) and play a role in species recognition (Endler, 1983). In cichlid fish, coloration covers this entire spectrum of functions, is important in nonsexually motivated communication, such as parent–offspring communication (Voss, 1977), in intraspecific intra- and intersexual selection (Hert, 1989; Evans & Norris, 1996) and in species recognition (Voss, 1977; Holzberg, 1978; Seehausen & van Alphen, 1998). There is great interspecific variation in coloration. Many cichlid species in the African Great Lakes have so recently diverged that molecular and morphological techniques have great difficulty in differentiating between them while they differ very much in coloration (e.g. Konings, 1988, 1995; Seehausen, 1996). Deutsch (1997) suggested that variation in hue is larger within than between species complexes among Lake Malawi cichlids, where the opposite is true for ecology.

The diverse coloration of closely related cichlid species suggests weak phylogenetic constraints, which, in combination with its intraspecific communication functions, makes colour variation a potential source of speciation caused by nonadaptive processes, e.g. self-escalating selection (Fisher, 1930; Lande, 1981). This hypothesis was first explicitly formulated for cichlid fish by Dominey (1984). Cichlid colour patterns are formed by the interaction of chromatophores, which are organized into five major systems (Baerends & Baerends-van Roon, 1950). System I chromatophores are small, equally distributed melanophores that, when expanded, cover the brighter colours produced by deeper laying chromatophores. Systems II and III are composed of larger melanophores that are situated deeper and form vertical bars (II) and longitudinal bands (III). System IV has been described in only one species. Systems V and VI consist of guanocytes (V) and erythrophores and allophores (VI), building up various hues from blue to red.

Recent studies strongly suggest that sexual selection plays a dominant role in moulding male nuptial coloration in the most species-rich cichlid lineage, the haplochromines (Deutsch, 1997; Seehausen *et al.*, 1997). These nuptial colours are formed by chromatophore systems V and VI. Less is known about the evolutionary forces that determine the evolution of the other

chromatophore systems. While systems V and VI are thought to be under hormonal control, the melanophores of systems II and III are under control of the central nervous system and can in different species be instantaneously expanded or contracted to varying degrees (Voss, 1977), suggesting that systems II/III and V/VI evolve under different kinds of selection regimes. Several ethological studies have been carried out on cichlid melanin stripes (Heiligenberg *et al.* 1972; Nelissen, 1975; Baerends, 1984; Baerends *et al.*, 1986), but the only comparative study (Voss, 1977) pre-dates the development of molecular and modern phylogenetic techniques. In that study of African riverine cichlids, Voss (1977) aimed to identify the optical language cichlids employ by context-dependent display of different elements in their melanin patterns. He found that members of East African lineages possess much less language than West African cichlids because of reduced chromomotor flexibility. This is consistent with the experience of taxonomists that most endemic species of the East African Great Lakes are well characterized by distinct and taxon-specific melanin patterns (Barel *et al.*, 1977; Eccles & Trewavas, 1989). At the same time, interspecific diversity in these patterns, which can consist of vertical, horizontal and cross-head stripe- and bar-like elements, is particularly large in Great Lake cichlids. As if superimposed on the other pigmentation, these patterns stand out like black script on coloured paper.

We wanted to identify the selective forces and constraints that explain the interspecific diversity of stripe patterns in East African lake cichlids, and whether they are similar to those which affect nuptial coloration, and have played a central role in the rapid diversification of lake cichlids (Deutsch, 1997; Seehausen *et al.*, 1997; Galis & Metz, 1998). We assemble published information on phylogenetic relations and coloration. We reconstruct the evolution of melanic stripe patterns and the origin of male nuptial coloration using parsimony analysis (PA), test for associations between coloration elements, and for such between coloration and ecology/behaviour by phylogenetic regression (PR), and for associations between colour pattern evolution and speciation by sister species contrasts (SC). We use our data to test six predictions, yielded by Dominey's hypothesis that cichlid coloration is little constrained and evolves rapidly under sexual selection with speciation as a by-product (Dominey, 1984; McElroy *et al.*, 1991; Deutsch, 1997; Seehausen *et al.*, 1997).

- 1 If melanic stripe pattern is phylogenetically unconstrained, changes should often occur at the tips of the phylogenetic tree.
- 2 If it is genetically/developmentally unconstrained, different stripes should evolve independently.
- 3 If it is ecologically unconstrained, stripes should evolve independently of ecology and behaviour.
- 4 If melanic stripe pattern, or some part of it, evolves primarily under sexual selection, the origin of such

stripes should be associated with the evolution of the mating system, or they should change faster in cichlids with mating systems that are conducive of sexual selection.

5 If nuptial coloration is primarily sexually selected as other studies make likely (Deutsch, 1997; Seehausen *et al.*, 1997), it should arise in association with mating systems that are conducive of sexual selection pressures.

6 If diversifying sexual selection plays an important role in speciation, lineages under strong sexual selection should speciate more often. Consequently, sister species in such lineages should differ in sexually selected traits more often than sister species in other groups, but less often in traits, the evolutionary pace of which is unaffected by sexual selection.

Materials and methods

The phylogeny

To obtain working phylogenetic hypotheses, we constructed two supertrees from published molecular estimates of phylogenetic relationships amongst East African cichlids. Published estimates include one for the taxon Haplochromini (the cichlids of Lakes Malawi and Victoria, and those of East African rivers; Meyer *et al.*, 1994), one covering all the other taxa (Nishida, 1997) and an alternative estimate for some of these other taxa and the basal branching order (Sturmbauer & Meyer, 1993; Meyer *et al.*, 1994; Sturmbauer *et al.*, 1994; subsequently referred to as the Meyer *et al.* -estimate). We combined the single estimate for the Haplochromini with the most detailed of the estimates available for the other taxa (Nishida, 1997), giving a strict supertree, *sensu* Sanderson *et al.* (1998), with consensus with the original trees (complete internal consensus).

To test for the effect of discrepancies between the estimates of Nishida and Meyer *et al.* we constructed another supertree that takes account of these discrepancies. We did this by using the basal branching sequence of the Meyer *et al.* estimate with their terminal resolution for those taxa that were covered, and terminal resolution of the Nishida estimate for taxa that were represented in the Meyer *et al.* estimate by a single taxon only. We carried out separate analyses on each of the two supertrees. This calls attention to the effects of discrepancies in estimates of phylogeny which would not be apparent when using a single strict consensus tree. Combining trees could be done by eye because they generally had only two taxa in common. The phylogenies are available on request from the corresponding author.

Meyer *et al.*'s (1994) phylogenetic estimate for the Haplochromini is based on the nucleotide sequence in 440 base pairs (bp) of the mitochondrial DNA (mtDNA) control region. Owing to their evolutionary youth, no internal molecular phylogenetic resolution is available for the Lake Victoria species flock, and only a little for the

Lake Malawi species flock. Nishida's (1997) estimate for the other taxa is a neighbour-joining tree derived from Rogers' genetic distances on the basis of data from 21 allozyme loci for 70 species of Lake Tanganyika cichlids. It includes all tribes, five of which were resolved to species level. Nishida's tree agrees well with a DNA-based likelihood tree based on the complete nucleotide sequence (1047 bp) of the NADH dehydrogenase subunit 2 gene obtained by Kocher *et al.* (1995) – but gives finer taxonomical resolution. The alternative estimates of Sturmbauer & Meyer (1993) and Sturmbauer *et al.* (1994) are consensus trees of parsimony and neighbour-joining analyses based on the nucleotide sequence in two segments (cytochrome *b* and control region) of the mitochondrial genome of 44 species. All tribes of Lake Tanganyika cichlids were included in their study; two were resolved to species level.

The colour data

Barel *et al.* (1977) provide an overview of common elements in the melanin patterns of East African cichlids. We studied all head markings and three common flank markings: vertical bars, and midlateral and dorsolateral stripe (Fig. 1). Other elements of flank markings, such as blotches, are autapomorphies of only a few groups and we did not consider them. Using the definitions and terminology of Barel *et al.* (1977), we collected information on melanin patterns of the taxa in the phylogeny from the following publications: Voss (1977; *Oreochromis*, *Astatotilapia burtoni*), Greenwood (1981a; Lake Victoria taxa), Ribbink *et al.* (1983; Lake Malawi taxa), Staeck (1985; Lake Tanganyika taxa), Herrmann (1987; Lake Tanganyika taxa), Konings (1988; Lake Tanganyika taxa 1995; Lake Malawi taxa), Eccles & Trewavas (1989; Lake Malawi taxa), Stiassny (1989; *Tylochromis*), Axelrodt (1993; *Oreochromis*, riverine *Lamprologus*), Skelton (1993; *Serranochromis*), Snoeks (1994; Lake Kivu taxa), Turner (1996; Lake Malawi taxa), Seehausen (1996; *Astatoreochromis*, *Astatotilapia bloyeti*, Lake Victoria taxa). All individuals of the respective taxa depicted in these monographs were scored. Where information on character states were ambiguous, the data set was further complemented by information from other publications (Fig. 2, Appendix 1). Information on flank coloration was available for all 107 taxa represented in the phylogeny. Additionally, we collected melanin pattern information for the major West and North African cichlid lineages (Tilapiines, Chromidotilapiines, Tylochromines), neotropical cichlids, Indian/Malagasy cichlids (Etoplines, Ptychochromines) and wrasses (Labridae), the marine outgroup to the family cichlidae. The literature sources that we used are identified in the captions to Fig. 3 and listed in Appendix 1.

Generally, scores are based on photographs of living fish, mostly photographed in their natural habitat. Only

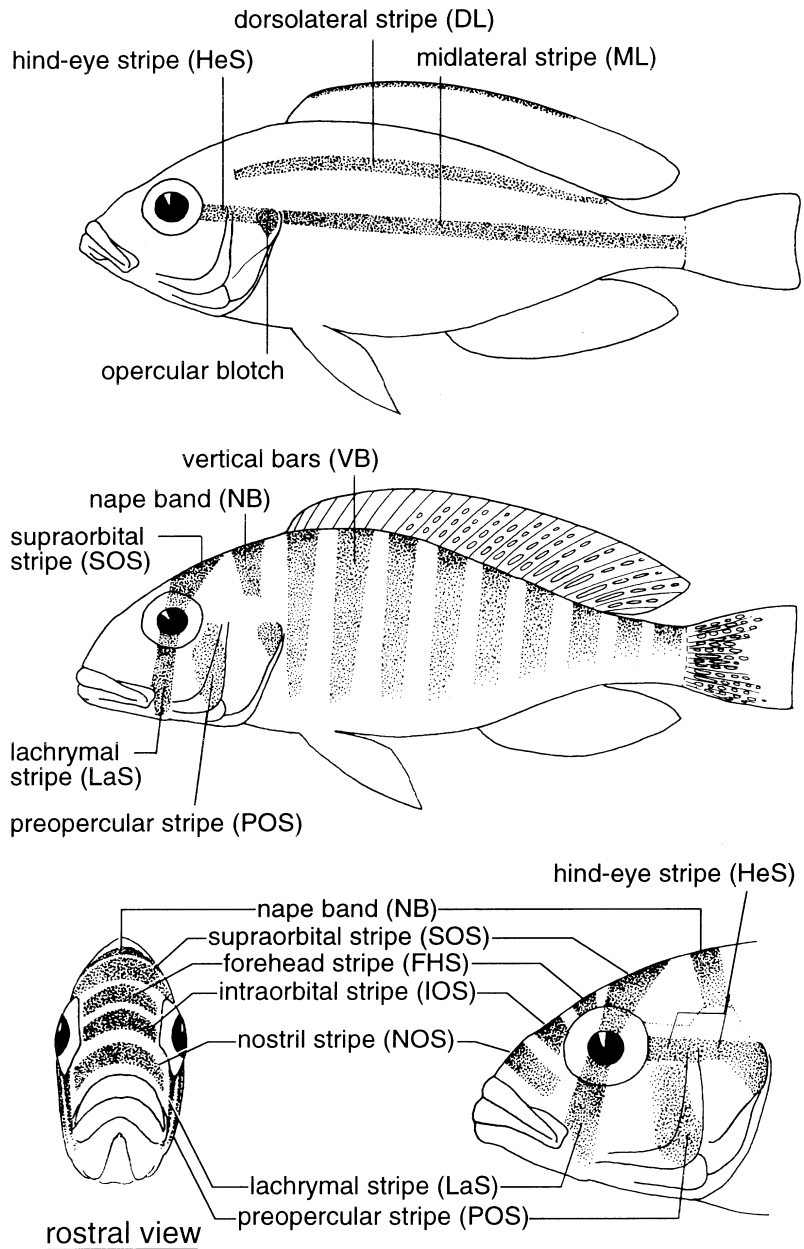


Fig. 1 The melanin markings of East African cichlid fish, considered in this study. Revised after Barel *et al.* (1977).

in exceptional cases where photographs were not available, drawings, supported by taxonomical descriptions, were used (indicated by an asterisk in Fig. 2). Because head markings can be indistinct on photographs, taxa were scored as having a head marking if it was visible on at least one photograph. Drawings were used to positively identify head markings only because some taxonomists or artists did not consider these markings on their drawings. Variation in the presence of flank markings was scored as polymorphism. We scored a maximum of two fishes from photographs that show larger groups of fish to avoid bias by photographs showing a shoal of fish

from the same spot in the same situation. We used the same publications to determine whether a taxon was sexually mono- or dimorphic (male nuptial coloration) for nonmelanic coloration. We excluded pictures of fishes in fright coloration and entirely melanic and xanthoristic (so called orange-blotched) fish because neither nuptial coloration nor melanic stripes are expressed in such individuals. Sample sizes are indicated behind each taxon in Fig. 2. Sample sizes per terminal taxon varied between 1 (4 taxa) and 563 individuals scored, with a median of 4 and an interquartile range from 3 to 7. The very large sample sizes were those for polytypic terminal taxa of the

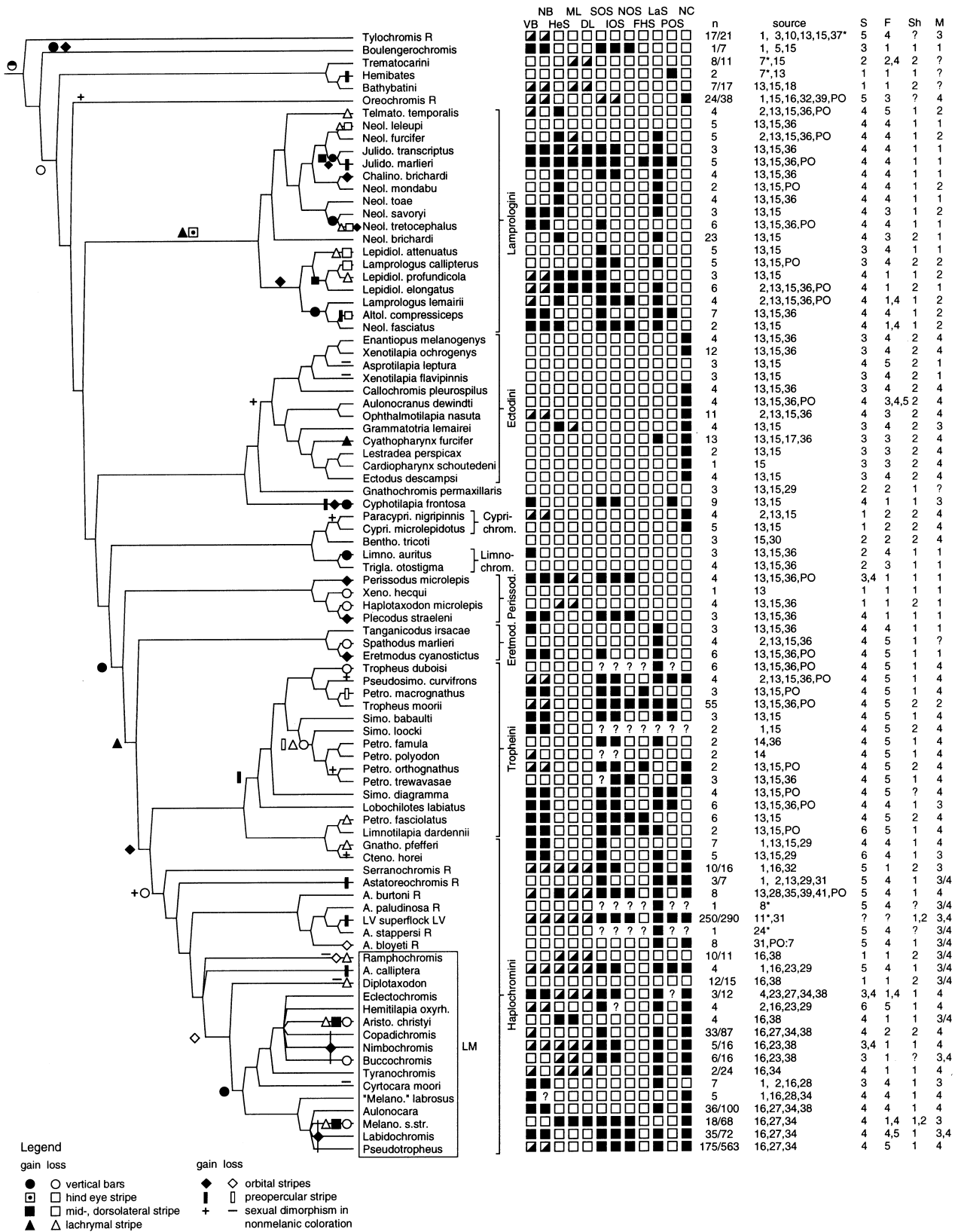
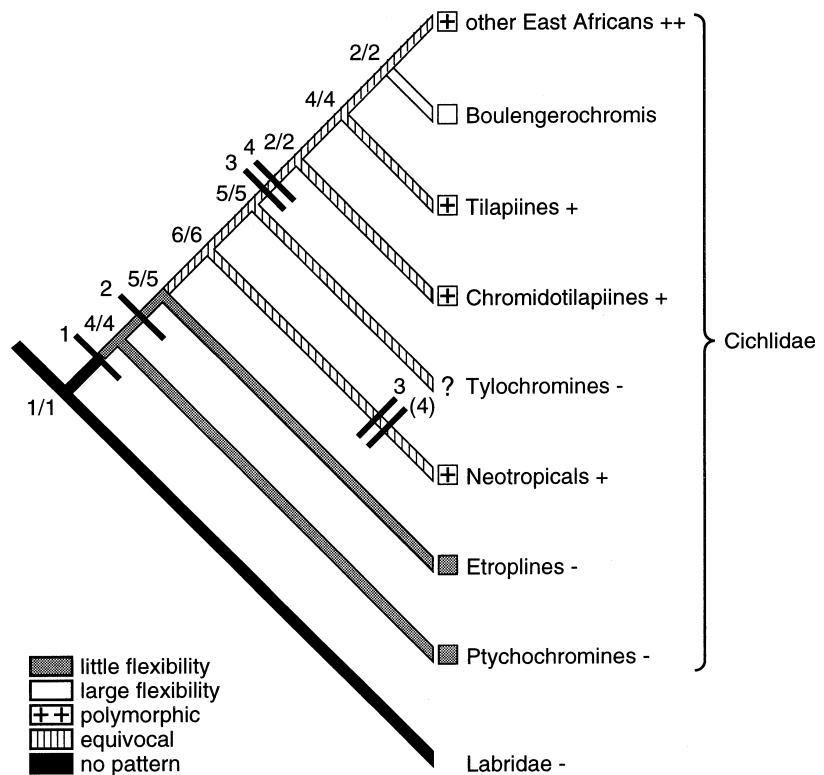


Fig. 2 The reconstructed history of the melanic stripe patterns (vertical bars, longitudinal stripes, orbital stripes, lachrymal stripe, preopercular stripe) and the origin of male nuptial coloration in the East African cichlid radiation. Strict supertree with character states unordered, and polymorphisms in polytypic terminal taxa resolved by 75% majority rule. Presence (black) and absence (white) of individual colour characters are indicated in boxes behind each taxon (half black = polymorphic, ? = unknown). *n* = sample size (species examined/individuals examined in case of polytypic taxa). Source refers to numbers in Appendix A (* based on drawings), PO = additional personal observations in the natural environment (photographs in the collection of the authors), not included in sample size except in *A. bloyeti* where seven specimens have been inspected. The symbol for the hind eye stripe refers to the condition without mid- and dorsolateral stripes. Character states in ecology and behaviour are given numerals. S substrate: 1 pelagial, 2 mud bottom, 3 sand bottom, 4 rocks, 5 rivers, 6 vegetation zones; F food: 1 fish, 2 zooplankton, 3 detritus/phytoplankton, 4 macroinvertebrates, 5 epilithic/epiphytic algae; Sh shoaling: 1 not shoaling, 2 shoaling; M mating system: 1 monogamy, 2 harem polygyny, 3 scramble polygyny, 4 promiscuity, 3/4 either 3 or 4. Abbr. R river-dwelling, LM endemic to Lake Malawi, LV endemic to Lake Victoria (all others are endemic to Lake Tanganyika). Genus names: *Neol.* = *Neolamprologus*, *Lepidiol.* = *Lepidiolamprologus*, *Altol.* = *Altolamprologus*, *A.* = *Astatotilapia*. To all other abbreviations of genus names should be added *chromis* (*Telmato.* = *Telmatochromis*, etc.). Other abbreviations as in Tables 1 and 2.

Fig. 3 The origin of major melanic stripes and melanophore chromator flexibility mapped on a consensus tree of the family cichlidae. Tree based on Oliver (1984), Stiassny (1991), Lippitsch (1995), Sueltmann *et al.* (1995), Zardoya *et al.* (1996) and Strelman & Karl (1997). Numerals at nodes indicate the number of studies that found a node and that of all studies that considered the lineages involved. Numerals on the character bars: 1 irregular lateral blotches, 2 vertical bars, 3 midlateral stripe, 4 interorbital stripe. -, +, ++ little, large, very large interspecific diversity in melanin pattern. All lineages downstream of a character bar contain taxa with that character, but not exclusively. Character 4 is restricted among neotropical cichlids to a few lineages but is found in all African lineages downstream of the bar. Ptychochromines are a Malagasy clade, Etroplines are an Indian/Malagasy clade, Labridae (wrasses) are the marine outgroup to the cichlids. Sample sizes (specimens/species) and data sources are as follows: Labridae 75/61 (sources 6, 40); Ptychochromines 34/12 (1, 4, 19, 20, 22, 25, 29); Etroplines 29/10 (1, 2, 4, 12, 19, 20, 22, 25, 28); Neotropicals 455/288 (1); Tylochromines 24/18 (1, 10, 37); Chromidotilapiines 84/25 (1); Tilapiines 88/61 (1); Boulengerochromis 7/1 (1, 5, 15); other East Africans as in Fig. 2.



Lake Malawi and Victoria flocks. Sample sizes on the major part of the tree (the Lake Tanganyika endemics) range from 1 to 55 with a median of 4 and an interquartile range from 2.5 to 5.5. Photographs of 1764 specimens were inspected for the East African phylogeny. Photographs of 697 specimens of other cichlid clades from all across the phylogeny were inspected, and drawings and photographs of 75 specimens of wrasses (Labridae).

Ecological and behaviour data

We collected data on habitat and diet, and on social and mating system. These were the only ecological and

behaviour variables available for a substantial proportion of the taxa considered. We differentiate between six habitat types (rivers, rocky lake regions, macrophyte-rich lake regions, sandy lake regions, muddy lake regions and the pelagic zone of lakes) and five diet categories (epiphytic/epilithic algae, macroinvertebrates, phytoplankton/detritus, zooplankton, and fish). Diet categories were chosen such as to represent the major feeding modes that occur among cichlids: benthic feeding on attached nonmovable prey (scraping, pulling), benthic feeding on movable prey (picking, pulling), pelagic and benthic feeding on loose nonmovable prey (sucking), pelagic feeding on small weakly movable prey (sucking), and hunting of evasive prey (Liem, 1980; Yamaoka, 1991).

We classified taxa as shoaling or not shoaling. Finally we differentiate between four mating systems (Kuwamura, 1997) that we use as measures of the potential strength of sexual selection by female choice. The latter is minimum in monogamy because the durable pairbond constrains the number of females that can mate with more attractive males. In harem polygyny, these constraints are somewhat relaxed because more attractive males can be mated by several females, the size of the harem being constrained by the males' ability to monopolize it. Temporal constraints on multiple matings of more attractive males are further relaxed in scramble polygyny (NT-polygyny in Kuwamura, 1997). There is no bond between the sexes before or after spawning, and males do not defend territories but follow females and may monopolize them by keeping other males out of reach. The number of females an attractive male can mate with is hardly limited temporally. Finally, in promiscuity, where males lek or otherwise defend territories that are visited by females, the potential strength of sexual selection is at a maximum (West-Eberhard, 1983; Dominey, 1984). Not only is the number of females that can mate with a more attractive male unlimited, but females sample several males before choosing one, and may have their clutches fertilized by more than one male (Kellogg *et al.*, 1995).

Data on ecology and social behaviour are from: Ribbink *et al.* (1983; Lake Malawi taxa), Konings (1988; Lake Tanganyika taxa, 1995; Lake Malawi taxa), Axelrodt (1993; *Oreochromis*, *Tylochromis*, riverine *Lamprologus*), Skelton (1993; *Serranochromis*), Spreinat (1995; Lake Malawi taxa), Turner (1996; Lake Malawi taxa), Seehausen (1996; *Astatoreochromis*, *Astatotilapia bloyeti*, Lake Victoria taxa), Hori (1997) and Yamaoka (1997) (both Lake Tanganyika taxa). Data on mating system are from Kuwamura (1997; Lake Tanganyika taxa), Herrmann (1987; *Eretmodus cyanostictus*), Skelton (1993; *Serranochromis*), Konings (1995; Lake Malawi taxa) and our own observations (*Astatoreochromis*, *Astatotilapia bloyeti*, Lake Victoria taxa).

Tests for correlated evolution by phylogenetic regression

We tested whether each melanin marking was correlated with the evolution of others, with ecological or with behaviour characteristics using the phylogenetic regression (PR) (Grafen, 1989). PR partitions the variance in terminal taxon characters into linear contrasts between sister taxa either side of each node on the tree. The contrasts are phylogenetically independent and are analysed using standard linear modelling techniques except that regression equations are always forced through the origin (Felsenstein, 1985; Burt, 1989; Harvey & Pagel, 1991; Garland *et al.*, 1992). Prior to calculating the contrasts, PR calculates appropriate branch lengths for a given tree topology using a maximum likelihood procedure

(Grafen, 1989). The result is a parameter, *Rho*, whose value is >1 when branch lengths are longest at the base of the tree, and <1 when branch lengths are greatest at the tips of the tree. The value of *Rho* may give an indication of where character changes are most likely to have occurred on the tree.

We coded melanin stripes and male nuptial coloration categorically as present (1) or absent (0) in a taxon and investigated their association with other stripes, nuptial coloration, ecological and behaviour variables coded as factors. Polymorphisms in the presence/absence of melanin markings were coded as present, except in the huge and diverse terminal taxon 'Lake Victoria superflock' and in the also very large taxa *Tylochromis* and *Oreochromis* that are distributed with many species across a wide range of ecological conditions. Polymorphisms in these taxa were coded as unknown. Polymorphisms in ecology were coded as unknown, except in polytypic terminal taxa that were given one state for habitat or diet if it occurred in 75% or more of the species. We did not resolve polymorphisms in shoaling behaviour but coded them as unknown because available data were not detailed enough. The only polymorphisms in the mating system were polytypic terminal taxa with scramble polygyny and promiscuity. Because available data on these and on other polygamous haplochromine taxa were not detailed enough, we lumped the two forms of polygamy under the term promiscuity.

Current comparative tests for investigating the evolution of categorical variables suffer from a number of problems and inadequacies (Ridley & Grafen, 1996), and PR is used here as one of the better performing, if imperfect, available applications (Grafen & Ridley, 1996). It should be noted that, because the phylogenetic data make it impossible to conduct this analysis entirely at the species level, some radiations are assigned a single terminal state and hence some of the variation between closely related species is not considered. When more phylogenetic data become available, it will be interesting to re-examine the results of our analysis.

Reconstruction of ancestral states by parsimony

We used MacClade 3.04 (Maddison & Maddison, 1992) to reconstruct the most parsimonious character states of tree nodes. Before coding characters, we studied their distribution. If one stripe was always associated with another stripe, we coded them as different states of one character. We first applied Fitch parsimony (Fitch, 1971; Hartigan, 1973), assuming an unordered transformation type. Second, we applied Wagner parsimony (Farris, 1970; Swofford & Maddison, 1987), assuming an ordered transformation type for multiple characters. In both cases the parsimony algorithms consider three sources of information that converge at a node and indicate its state (Swofford & Maddison, 1987): those from the right and left descendants of a node, and that from the part of

the tree below the node. For unordered character states, the algorithm chooses those states occurring in the greatest number of the three sets of most parsimonious solutions for a node. For ordered character states, the algorithm takes into account the number of steps needed for each state transformation across the node. In the case of binary characters, polymorphic states are treated just like unknown states.

Where branches in the available phylogeny terminated on a single representative of a polytypic genus (several Lake Malawi genera, *Serranochromis*, *Astatoreochromis*, and branches deep in the phylogeny), all character states were considered that were found in that genus. Hence, such polytypic terminal taxa were treated as polymorphic. Terminal polymorphism is equivalent to character polytomy. To resolve these polytomies we used two options: since the internal phylogeny of the polytomies was unknown, we first considered variation within the taxon as represented by a soft polytomy. Character states are then equally important, independent of their frequency of occurrence. Secondly, we applied a 75% majority rule, giving the polytypic taxon the character state that was represented by at least 75% of its constituent taxa. In determining the 75% majority character state, established taxonomical species complexes (Ribbink *et al.*, 1983; Konings, 1995) within the taxon were treated as only one independent data point (Deutsch, 1997), the state of which was again determined by 75% majority. We preferred 75% majority over a hard polytomy option to reduce the impact of chance bias in large terminal taxa of which generally not all species were known.

For each tree MacClade calculates a consistency index and a retention index. The consistency index (Kluge & Farris, 1969; Farris, 1989) measures how much the most parsimonious number of steps is greater than the minimum conceivable number on any tree with the same data set for extant taxa (as a proportion where 0 = infinitely more, 1 = minimum). The CI is therefore a measure of homoplasy in the reconstructed history of character evolution, ranging from entirely homoplastic (0) to no homoplasy (1). The retention index (Archie, 1989a,b; Farris, 1989) measures whether the observed number of steps reconstructed on the tree in question is closer to the minimum or maximum possible (as a proportion where 0 = maximum, 1 = minimum). The RI is therefore a measure of how readily characters reverse their states. We did two separate analyses: one on the strict supertree, and one on the alternative supertree with the basal branching order and the resolution of the tribes Lamprologini and Ectodini based on the Meyer *et al.* estimate.

Sister species contrasts

We performed comparisons between pairs of sister-species at the tips of the phylogeny. Such taxa share very recent common ancestors and, hence, reveal much about current evolutionary processes, associated with

speciation, whilst interpretation of the data is not hampered by uncertainties about character states deeper in the evolutionary tree. To compare the frequency of speciation-associated changes in melanic stripes, non-melanic coloration (hue) and ecology, we counted cases of stasis and change in all traits across sister species and compared the stasis/change ratios obtained for different traits by means of two by two contingency tables.

All sister species pairs were from Lake Tanganyika. The Lake Malawi and Victoria radiations have occurred so recently that incomplete mitochondrial DNA lineage sorting among the species limits applicability of mtDNA sequencing to species-level phylogenetic reconstruction (Moran & Kornfield, 1993). All species in Lakes Malawi and Victoria are promiscuous female mouthbrooders and the vast majority possess male nuptial coloration (Fryer & Iles, 1972; Greenwood, 1981; Konings, 1995; Seehausen, 1996). In contrast, clades in Lake Tanganyika are diverse in mating systems and, hence, in their exposure to sexual selection by mate choice.

To test whether, and in which characters, the rate of evolutionary change might be affected by the strength of sexual selection, we compared sister species stasis/change ratios in weakly sexually selected (pair bonding and harem forming) species with those in strongly sexually selected (promiscuous) species. After having identified traits that evolve faster in promiscuous than in pair bonding/harem-forming systems, we tested the related prediction that, within the promiscuous species, these traits change faster than other traits but within the pair bonding/harem-forming species do not change faster than other traits. We did all tests with the lamprologine and ectodine sister species pairs of the Meyer *et al.* estimate (Sturmbauer & Meyer, 1993; Sturmbauer *et al.*, 1994) and those of the Nishida-estimate (Nishida, 1997) separately. The latter tests do not dispense with the possibility that properties of clades other than mating system could control colour change, and were performed because current phylogenetic information makes it impossible to map species richness, speciation rates or rates of colour change onto clades (see Purvis, 1996).

Results

Phylogenetic regression

Associations between melanin markings

Regression coefficients ranged from -0.16 to $+0.83$ (Table 1). Among 110 possible associations were five that were significantly positive on only one of the two trees and 17 that were significantly positive on both trees. The strongest associations were found between the dorsolateral and the midlateral stripe, the nape band and vertical bars, the supraorbital and the interorbital stripe, the midlateral and the hind-eye stripe, and the nostril and the interorbital stripe.

Table 1 Phylogenetic associations among melanin stripes, and between stripes and male nuptial coloration. Association coefficients are based on the strict supertree shown in Fig. 2. Associations based on the alternative supertree, with the Meyer *et al.*-estimate, were in most cases similar. Bold printed associations were significant on both supertrees; associations followed by an asterisk were significant only on the supertree with the Meyer *et al.*-estimate. VB vertical bars, NB nape band, SOS supraorbital stripe, IOS interorbital stripe, NOS nostril stripe, FHS forehead stripe, POS preopercular stripe, LaS lachrymal stripe, ML midlateral stripe, DL dorsolateral stripe, HeS hind-eye stripe, NC male nuptial coloration, V vertical elements, L longitudinal elements, C cross-head stripe. Significance level is $P < 0.05$ after correction by sequential Bonferroni method (Rice, 1989).

Independ.	VB V	NB V	SOS V	IOS C	NOS C	FHS C	POS V	LaS V	ML L	DL L	HeS L	NC
Dep.												
VB V		0.75	0.55	0.41	0.31	0.38	0.26	0.17	0.02	0.30	0.05	-0.06
NB V	0.72		0.57	0.40*	0.23	0.45	0.29	0.12	-0.09	0.11	-0.15	0.09
SOS V	0.38	0.41		0.65	0.46	0.18	0.30	0.12	0.02	0.21	0.01	0.09
IOS C	0.30*	0.30*	0.74		0.60	0.38	0.18	0.19	0.00	0.14	0.24	0.02
NOS C	0.18	0.15	0.35	0.44		0.53	0.09	-0.08	0.06	0.08	0.21	0.07
FHS C	0.09	0.12	0.09	0.13	0.22		0.04	-0.03	0.04	0.05	0.00	-0.07
POS V	0.11	0.15	0.23	0.15	0.02	0.11		0.25	-0.02	0.00	-0.05	-0.04
LaS V	0.12	0.09	0.18	0.20	-0.16	-0.11	0.41		-0.06	0.01	0.10	0.17
ML L	0.02	-0.07	0.02	0.01	0.08	0.09	-0.03	-0.04		0.83	0.61	0.08
DL L	0.17	0.06	0.14	0.09	0.09	0.11	0.08	0.06	0.61		0.34*	0.05
HeS L	0.04	-0.10	0.01	0.17	0.24	0.01	-0.06	0.09	0.63	0.43*		0.07
NC	-0.02	0.07	0.07	0.00	-0.02	-0.15	0.01	0.11	0.05	0.04	0.05	

If stripes are assigned, according to their orientation relative to the fish body axis, to three groups: longitudinal (L), vertical (V) and cross (C) stripes (Fig. 1), it becomes apparent that associations between different longitudinal stripes and between different vertical stripes are more common than associations between stripes of different orientation (Table 1). The two ventrally positioned vertical stripes (POS, LaS) behave independently of the dorsally positioned vertical stripes (VB, NB, SOS). Cross stripes are associated with vertical, or with other cross stripes. This result makes possible the existence of genetical/developmental constraints on certain stripe combinations but demonstrates considerable degrees of freedom in melanin pattern evolution. It is therefore consistent with the hypothesis of weakly constrained colour evolution.

Ecological and behaviour correlates of colour evolution

Phylogenetic regressions demonstrate several ecological and behaviour constraints on melanin pattern (Table 2). The evolution of vertical bars and supraorbital stripes is associated with habitat affiliation. Presence or absence coincide with switches between structured littoral habitats (rocky and vegetated lake shores, rivers) and unstructured habitats, though the significance of these results is somewhat sensitive to tree topology (Table 2b).

The evolution of the midlateral stripe is associated with feeding mode. Presence or absence correlate positively with presence or absence of piscivory (Table 2b). Shoaling has positive effects on the evolution of the two large longitudinal stripes and, albeit with a shallower slope, on the evolution of the forehead stripe (Table 2a). Controlling for the effects of shoaling, feeding mode retains its significant effect on midlateral stripe. Significance of the

association even increases (Table 2b). In contrast, the effects of shoaling on the midlateral stripe are no longer significant if those of feeding mode are controlled for (Table 2a). None of the melanin stripes is associated with the strength of sexual selection as measured by mating system. These results on melanin pattern are at odds with the hypothesis of weakly constrained and nonadaptive evolution of coloration.

On the other hand, the origin of male nuptial coloration is associated with mating system evolution, consistent with the hypothesis of evolution under sexual selection (Table 2a,b). Presence or absence correlate with transitions from monogamy/harem polygyny to promiscuity and the reverse. The origin of nuptial coloration is associated also with habitat use. Presence or absence coincide with switches between littoral hard bottom and offshore soft bottom habitats, though the significance of this result is somewhat sensitive to tree topology (Table 2a,b).

We could not calculate associations between mating system and ecology or between ecological variables because available phylogenetic comparative methods do not allow the calculation of associations between two categorical variables with multiple states. However, the evolution of shoaling behaviour (a binary character) is significantly associated with feeding mode, shoaling evolved most often in association with planktivory and least often in association with benthic feeding (Table 2a,b).

Rho

The values of *Rho* yielded by phylogenetic regressions are low (Table 3a) and suggest that transitions in coloration have often occurred between closely related species.

Table 2 Phylogenetic associations between coloration and ecology and behaviour
 A: all associations; where the strict supertree and the alternative supertree yielded different results, both are given separated by “/” (strict/alternative).
 B: coefficients of significant associations (based on strict supertree in top row, on alternative supertree in bottom row); pelagial, fish, and monogamy are used as the standard against which others are compared.

Abbr.: Sub, substrate, Feed, feeding mode, Sh shoaling, F_(Sh) feeding mode, controlling for shoaling behaviour, Sh_(F) shoaling behaviour, controlling for feeding mode, pelagial, veget. vegetation zones, zoo, zooplankton, phy, phytoplankton/detritus, mac, macroinvertebrates, epi, epilithic/epiphytic algae, mon, monogamy, poly, harem polygyny, prom, promiscuity, midlat, str._(Sh) effects of shoaling on midlateral stripe are controlled for, dorsolat, str._(F) effects of food on dorsolateral stripe are controlled for, ns not significant, * significant, P < 0.05. V, L, C as in table 1.

A indep.:	Sub.	Feed.	Sh	F _(Sh)	Sh _(F)	Mating system	B	pela.	rivers	mud	sand	rock	veget.	F	d.f.	P
dep.:																
vertical bars	*/ns	ns	ns			ns	vertical bars	0	0.26	-0.07	0.11	0.52	0.73	2.43	5,49	<0.05
nape band	ns	ns	ns			ns	supraorbit. str	0	0.22	0.12	0.05	0.35	0.72	2.11	5,50	<0.1
supraorbital str.	ns/*	ns	ns			ns		0	0.77	0.11	0.66	0.67	0.85	2.35	5,27	<0.1
lacrimal str.	ns	ns	ns			ns	V	0	0.55	0.12	0.52	0.68	0.84	2.97	5,26	<0.05
preopercular str.	ns	ns	ns			ns		0	0.68	-0.19	0.37	0.52	0.65	2.68	5,45	<0.05
V	*/ns	ns	ns			ns	nuptial col.	0	0.53	0.08	0.44	0.50	0.77	2.03	5,46	<0.1
						ns		0	0.63	-0.46	0.29	0.18	0.54	2.60	5,29	<0.05
						ns		0	0.49	-0.04	0.29	0.18	0.49	2.14	5,26	<0.1
midlateral str.	ns	*/ns	*	*	ns	ns	fish		zoo.	phy.	mac.					
dorsolateral str.	ns	ns	*		ns/*	ns	midlateral str.	0	-0.31	-0.56	-0.45	-0.57		3.42	4,35	<0.05
hind-eye str.	ns	ns	ns		ns	ns		0	-0.33	-0.51	-0.40	-0.53		2.61	4,29	<0.1
L	ns	*	ns		ns	ns	midlat. str. (Sh)	0	-0.38	-0.57	-0.38	-0.51		3.75	4,45	<0.05
						ns		0	-0.32	-0.50	-0.30	-0.45		2.81	4,39	<0.05
interorbital str.	ns	ns	ns		ns	ns	L	0	-0.63	-0.48	-0.51	-0.56		2.68	4,37	<0.05
nostril str.	ns	ns	ns		ns	ns		0	-0.68	-0.57	-0.54	-0.67		2.81	4,30	<0.05
forehead str.	ns	ns	*		ns	ns	shoaling	0	0.41	0.19	-0.16	-0.15		2.69	4,37	<0.05
C	ns	ns	ns		ns	ns		0	0.15	0.15	-0.26	-0.19		3.25	4,36	<0.05
nuptial col.	*/ns	ns	ns		*	*	nuptial col.	0	0.07	0.69				8.58	2,23	<0.01
shoaling	ns	*			ns	ns		0	0.04	0.55				8.42	2,21	<0.01
							shoaling		mon.	poly.	prom.					
midlateral str.								0.24						4.61	1,39	<0.05
dorsolat. str.								0.30						7.27	1,34	<0.05
dorsolat. str. _(F)								0.27						5.58	1,30	<0.05
forehead str.								0.13						9.30	1,27	<0.05
								0.19						1.96	1,46	ns
								0.12						4.75	1,37	<0.05
								0.14						4.49	1,21	<0.05
														9.12	1,18	<0.05

Table 3 A. Estimates of branch lengths. Values of *Rho* for coloration characters yielded by phylogenetic regressions. st = strict supertree, al = alternative supertree.

	st	al		st	al
vertical bars	0.07	0.05	interorbital stripe	0.18	0.17
nape band	0.09	0.09	nostril stripe	0.07	0.10
midlateral stripe	0.08	0.17	forehead stripe	0.11	0.22
dorsolateral stripe	0.17	0.17	lachrymal stripe	0.14	0.18
hind-eye stripe	0.11	0.14	preopercular stripe	0.11	0.18
supraorbital stripe	0.22	0.21	nuptial coloration	0.41	0.39

Table 3 B. Estimates of homoplasy and character reversal. Consistency (CI) and retention (RI) indices of coloration, ecological and behaviour characters. Vertical elements exclude lachrymal and preopercular stripe which behaved independent from other vertical elements (see text). bin = binary character. Ensemble indices are printed in bold.

	Strict supertree				Alternative supertree			
	unordered CI	RI	ordered CI	RI	unordered CI	RI	ordered CI	RI
vertical elements	0.62	0.40	0.60	0.43	0.53	0.31	0.51	0.33
longitudinal elements	0.78	0.45	0.75	0.39	0.74	0.30	0.75	0.41
orbital stripes	0.23	0.34	0.13	0.49	0.25	0.38	0.14	0.52
lachrymal stripe (bin)	0.06	0.60			0.06	0.62		
preopercular stripe (bin)	0.10	0.18			0.10	0.25		
stripe tree	0.44	0.43	0.45	0.47	0.41	0.43	0.42	0.47
nuptial coloration (bin)	0.08	0.68			0.09	0.71		
substrate	0.38	0.47			0.37	0.37		
feeding	0.41	0.49			0.39	0.51		
ecology tree	0.40	0.48			0.38	0.45		
shoaling	0.12	0.46			0.12	0.44		
mating system	0.24	0.47	0.16	0.63	0.26	0.54	0.19	0.72
behaviour tree	0.19	0.47	0.15	0.59	0.20	0.50	0.17	0.65

This holds for melanic stripes in particular, while transitions between absence and presence of male nuptial coloration evolved slightly deeper in the tree.

Parsimony analysis

Reconstruction of character evolution

We discovered the following 100% associations between stripes: a nape band (NB) occurred only where vertical bars (VB) were present. A dorsolateral stripe (DLS) occurred only where a midlateral stripe (MLS) was present except in a single, difficult to interpret case (*Julidochromis transcriptus*). A midlateral stripe occurred only where a hind-eye stripe (HeS) was present. An interorbital stripe (IOS) occurred only if a supraorbital stripe (SOS) was present. A nostril stripe (NoS) and a forehead stripe (FHS) occurred only if an IOS was present, but NoS and FHS were not associated. After coding tied stripes as states of one character, six characters remained. Three were binary and could be absent (0) or present (1): opercular blotch (OpB), lachrymal stripe (LaS), preopercular stripe (POS). The other three were multiple: vertical elements (VE: absent, VB, VB + NB),

longitudinal elements (LE: absent/HeS/HeS + MLS/HeS + MLS + DLS/HeS + DLS) and orbital stripes (OS: absent/SOS/SOS + IOS/SOS + IOS + NoS/SOS + IOS + NoS + FHS/SOS + IOS + FHS).

Agreement between molecular phylogeny and melanin pattern is moderate (Table 3b ensemble indices, Fig. 2). Neither the discrepancies between the Nishida- and the Meyer *et al.*-phylogenetic estimates nor the two polytomy options had significant influence on the reconstruction of character evolution. Treating character states as ordered or unordered had little effect on the results too (Table 3b). We illustrate the results using the strict supertree with the Nishida-estimate (Fig. 2) because of its internal consensus, more detailed resolution and slightly higher consistency indices. We map characters as unordered on the tree because the underlying transformation model implicates fewer evolutionary steps. Only one character, the opercular blotch, was universally present and is obviously plesiomorphic of East African cichlids. We omitted it when calculating tree indices.

Below we summarize major phylogenetic trends, not considering apomorphies of individual species. Vertical bars are the most widely distributed character. They

occur with a patchy distribution in West African, neotropical and Indian/Malagasy cichlids too, and some riverine species without distinct patterns sometimes show faint traces of vertical bars. It is most likely that vertical bars are plesiomorphic in East African cichlids. Parsimony, however, suggests that distinct vertical bars have been lost early in the lacustrine radiations and have been regained in several small clades of the Lamprologini, and in one large clade of mouthbrooding cichlids [Perissodini–Eretmodini–Tropheini when based on Nishida (1997), Eretmodini–Tropheini when based on Sturmbauer & Meyer (1993)] within Lake Tanganyika. Within the latter clade they appear to have been lost upon emigration from the lake into rivers (*Serranochromis*, *Astatoreochromis*, *Astatotilapia*). These riverine haplochromines most likely lacked distinct melanin stripes on the flanks when they eventually colonized Lakes Malawi and Victoria. In both lakes distinct vertical bars were regained in clades that radiated in the littoral lake zones.

Longitudinal stripes, although found in West African and neotropical cichlids too, are in the East African radiation apomorphies of a few, mostly lacustrine clades (Fig. 2): the Bathybatini and Lamprologini in Lake Tanganyika (hind-eye stripe without flank stripe as a unique synapomorphy of Lamprologini), the riverine/lacustrine piscivorous genus *Serranochromis*, the piscivorous genus *Ramphochromis* in Lake Malawi, at least three other piscivorous genera of the Lake Malawi subflock A, the partly piscivorous genus *Melanochromis* of the subflock B, two largely piscivorous (*Prognathochromis* and *Harpagochromis*) and one macroinvertebrate-eating lineage (including *Paralabidochromis*, *Ptyochromis*, *Macropleurodus*, *Platytaeniodus* and *Hoplotilapia*) of haplochromines in Lake Victoria (not shown because no molecular phylogeny available).

Orbital stripes, outside the East African radiation found also in some West African and in some neotropical species, are apomorphies of a few lacustrine clades (Fig. 2). They show evolutionary trends that are similar to those seen in vertical bars, with the difference that orbital stripes were not lost when the ancestor of the haplochromines emigrated from Lake Tanganyika into rivers. The presence of orbital stripes appears to be a plesiomorphic condition in the species flocks of Lake Victoria and Malawi. The condition with interorbital stripe is a synapomorphy of the Tropheini–Haplochromini and of some lineages within the Lamprologini. The nostril stripe is rare and largely restricted to haplochromines. The forehead stripe is very rare and is restricted to some Tropheini and possibly some lamprologine species.

The lachrymal stripe is a synapomorphy of the Lamprologini and the large clade Eretmodini–Tropheini–Haplochromini. The lachrymal stripes clearly differ between the two clades. The lamprologine stripe runs obliquely from the ventral margin of the eye to the upper lip. A similar stripe is sometimes observed in one ectodine species. A similar stripe is known also from some West

African and neotropical cichlids. The haplochromine stripe runs more vertically from the eye downwards, passing just posterior to the maxilla, and reaching the ventral side of the head (Barel *et al.*, 1977). Apart from several phylogenetically isolated occurrences, the preopercular stripe appears to be synapomorphic for the major branch within the Tropheini in Lake Tanganyika, for the genus *Astatoreochromis*, and for the Lake Victoria haplochromines.

It is beyond the scope of this paper to discuss the evolution of ecological and behaviour traits but a few robust trends that emerged deserve mentioning. Most substrate affiliations and diets have evolved more than once within Lake Tanganyika (Fig. 2). Despite this, trees are moderately well supported by ecological consistency and retention indices (Table 3b). Transitions between sand and rock shores and between mud bottoms and the pelagic zone are more common than other transitions in substrate affiliation, and it is likely that the ancestors of the species that emigrated from the lake into East African rivers (haplochromines) and the Zaire river (lamprologines), were derived from rock-dwelling cichlids. The plesiomorphic feeding mode in East African cichlids is benthic feeding on macroinvertebrates. Most transitions occur from this to various derived modes. There are few transitions between derived modes and very few reversals from derived modes to benthic feeding on macroinvertebrates. Shoaling behaviour and mating systems have evolved more than once too, but the mating system has a somewhat higher retention index than other ecology and behaviour traits when its character states are considered as ordered. Particularly transitions between mating systems with mate bond and promiscuity rarely occur at the tips of the phylogenetic tree.

The ensemble consistency indices yielded by stripe pattern and by ecology are similar; that yielded by behaviour is lower. The ensemble retention indices are all similar. Individual ecology and behaviour characters yielded indices close to the respective ensemble indices, while both the amount of homoplasy (CI) and character reversal (RI) varied considerably among stripes (Table 3). Orbital stripes, lachrymal stripe and preopercular stripe yielded CIs that were much lower than those of flank markings and those of ecology. The RI of lachrymal stripe was higher, and that of preopercular stripe lower than those yielded by other characters. These results are consistent with the hypothesis of little constraint on the evolution of those stripes that are not associated with ecology.

Sister species contrasts

In Table 4, we show all sister species pairs that were found on the trees, state whether they are pair bonding/harem forming or promiscuous, and tell for any character whether the sister species differ in it. 'Change' in stripes means change between presence or absence, 'change' in hue means change between spectral colours. The only

Table 4 Stasis and change in sister species contrasts. M mating system: B pair bonding/harem forming, P promiscuous; VE vertical elements (excluding LaS, POS), LE longitudinal elements, OS orbital stripes, LaS lachrymal stripe, POS preopercular stripe, S substrate, F food (+ = change, - = stasis). ^N taxa of Nishida (1997) and ^S taxa of Sturmbauer & Meyer (1993), Sturmbauer *et al.* (1994) for the two tribes that were resolved to species level in both studies.

	M	VE	LE	OS	LaS	POS	Hue	S	F
Lamprologini									
<i>leleupi-furcifer</i> ^N	B	-	+	-	+	-	+	-	-
<i>transcriptus-marlieri</i> ^N	B	-	+	+	-	+	-	-	-
<i>brichardi-mondabu</i> ^N	B	-	-	+	-	-	-	-	-
<i>savoryi-tretocephalus</i> ^N	B	-	+	+	+	-	+	-	+
<i>callipterus-profundicola</i> ^N	B	+	+	+	+	-	-	-	+
<i>compressiceps-fasciatus</i> ^N	B	-	+	+	-	+	-	-	+
<i>marlieri-regani</i> ^S	B	+	+	-	-	-	-	-	-
<i>bifrenatus-vittatus</i> ^S	B	-	+	+	-	-	-	-	?
<i>wernerii-congoensis</i> ^S	B	+	-	-	-	-	-	-	?
<i>christyi-burgeon</i> ^S	B	-	+	?	?	?	-	+	-
<i>furcifer-brichardi</i> ^S	B	-	-	+	-	-	-	-	-
<i>calliurus-brevis</i> ^S	B	+	-	-	+	-	-	+	+
<i>calvus-compressiceps</i> ^S	B	-	-	-	-	-	-	-	-
<i>toae-cylindricus</i> ^S	B	+	+	+	-	-	+	-	-
Ectodini									
<i>melanogenys-ochrogenys</i> ^N	P	-	-	-	-	-	+	-	-
<i>dewindti-nasuta</i> ^N	P	+	-	-	-	-	+	+	+
<i>perspicax-schouteden</i> ^N	P	-	-	-	-	-	-	-	-
<i>tenuidentatus-leptura</i> ^S	B	+	-	-	-	-	-	+	+
<i>perspicax-longiventralis</i> ^S	P	-	-	-	-	-	+	+	+
<i>dewindti-descamps</i> ^S	P	-	-	-	-	-	?	+	+
<i>ventralis-schouteden</i> ^S	P	-	-	-	-	-	+	+	-
Cyprichromini									
<i>nigripinnis-microlepidotus</i>	P	+	-	-	-	-	+	-	-
<i>auritus-otostigma</i>	B	+	-	-	-	-	+	-	+
Perissodini									
<i>H. microlepis-P. straeleni</i>	B	+	+	+	-	-	-	+	-
Ectodini									
<i>marlieri-cyanostictus</i>	B	+	-	+	-	-	-	-	-
Tropheini/Haplochromini									
<i>macrogathus-moorii</i>	P	+	-	+	+	+	+	-	-
<i>famula-polyodon</i>	P	+	-	-	+	-	+	-	-
<i>orthognathus-trewavasae</i>	P	+	-	?	-	-	+	-	-
<i>fasciolatus-dardennii</i>	P	-	-	+	+	-	-	+	+
<i>pfefferi-horei</i>	P	-	-	-	+	-	+	-	-

character that changed more often among promiscuous sister species was hue (Table 5a). There was a tendency for lachrymal stripe to change more often too but this was not robust to different phylogenetic hypotheses. Some melanin stripes changed among promiscuous species even less often than among pair bonding/harem-forming species. This result suggests that the evolution of stripe pattern is not driven by sexual selection, but that of hue might be.

Among promiscuous sister species change in hue is more common than change in melanic stripes or in ecology. This is robust to different phylogenetic hypotheses. Among pair bonding/harem-forming sister species, in contrast, change in some stripes is more common than

change in ecology and in hue but these results are not robust to different phylogenetic hypotheses (Table 5b). These results agree with predictions yielded by the hypothesis that diversifying sexual selection upon hue plays an important role in speciation of promiscuous cichlids: speciation among them is more often associated with changes in hue than (i) with changes in stripe pattern or ecology, and (ii) in pair bonding/harem-forming species. The latter differ more often in stripe pattern.

Discussion

Our phylogenetic comparative tests of Dominey's hypothesis have shown: (1) that changes in melanic stripe

Table 5 A. Stasis/change ratios for melanin markings, hue and ecology in sister species contrasts of pair bonding/harem-forming and promiscuous species. VE vertical elements (excluding LaS, POS), LE longitudinal elements, OS orbital stripes, MP melanin markings pooled, Eco ecological variables pooled. Other abbreviations as in Table 1. A. Comparison between pair bonding/harem forming (B) and promiscuous (P) species. Number of cases in which sister species do not differ/number of cases in which they differ. P = significance level, ns = $P > 0.1$.

	Strict supertree			Alternative supertree		
	B	P	P	B	P	P
VE	5/4	4/5	ns	4/8	5/4	ns
LE	3/6	9/0	0.003	7/5	9/0	0.03
OS	2/7	6/2	0.03	6/5	6/2	ns
LaS	6/3	5/4	ns	10/1	5/4	0.07
POS	7/2	8/1	ns	11/0	8/1	ns
S	8/1	7/2	ns	8/4	5/4	ns
F	5/4	7/2	ns	7/3	6/3	ns
Hue	6/3	2/7	0.06	10/2	1/7	0.002
MP	23/22	32/12	0.04	38/19	33/11	ns
Eco	13/5	14/4	ns	15/7	11/7	ns

Table 5 B. Comparison between characters within a mating system. > changes are significantly ($P < 0.05$) more frequent in row than in column variable. Where the strict and the alternative supertree yield different results, both are given separated by '/' (strict/alternative).

	Pair bonding/harem forming species								Promiscuous species									
	LS	OS	La	POS	S	F	Hue	MP	Eco	LS	OS	La	POS	S	F	Hue	MP	Eco
VE	-	-	-/>	-/>	-	-	-/>	-	-/>	>	-	-	>/-	-	-	-	-	-
LE	-	-	-	-	>/-	-	-	-	-	-	-	<	-	-/<	-	<	-	-
OS	-	-	-	>	>/-	-	-	-	>/-	-	-	-	-	-	-	<	-	-
LaS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
POS	-	-	-	-	-/<	-	-	-	-/<	-	-	-	-	-	-	<	-	-
S	-	-	-	-	-	-	-	</-	-	-	-	-	-	-	-	</-	-	-
F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<	-	-
Hue	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	>	>
MP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

pattern have frequently occurred in the evolution of the East African cichlid fish; (2) that some stripes evolve independently, others are often, and some are always associated, and that natural selection acts differently upon different groups of stripes; (3) that some changes in stripe pattern are associated, with changes in resource utilization and/or behaviour; (4) that mating system evolution has little effect on stripe pattern evolution but is associated with the origin of male nuptial coloration, and that promiscuous sister species differ more frequently in hue but less frequently in stripe pattern than sister species with pair bond or harem formation. Below we discuss each of these results and what they tell us about the history of, and the selection forces that act upon coloration in the East African cichlid radiations.

I Parsimony analysis suggests that all kinds of melanin stripes have evolved more than once within Lake Tanganyika, and several have been lost again. The low values of ρ yielded by phylogenetic regressions suggest that much of the change in striping occurred between closely related species. Parsimony suggests that the major

transitions are associated with major changes in ecology, such as switches between rivers and lakes and between habitats within the lakes. Most riverine East African cichlids, from clades all over the phylogeny, such as *Oreochromis*, riverine *Lamprologus* and riverine haplochromines lack distinct melanin stripes, particularly on the flanks (Fig. 2). Members of the latter two diversely striped clades, which originated in Lake Tanganyika, left the lake and invaded central and East African river systems. Parsimony analysis tells us that upon leaving the lake they lost distinct flank striping and that most kinds of stripes that their lacustrine ancestors had possessed, evolved again within a very short time when the haplochromines literally exploded into various ecological niches upon invasion of Lakes Malawi and Victoria. Moreover, in both these lakes several unique kinds of melanin pattern arose that are not known from any other African cichlid (Eccles & Trewavas, 1989; Seehausen *et al.*, in press). Hence, there seems to be little historical constraint on melanin pattern evolution in East African cichlids.

2, 3 As expected from some of the foregoing, flank stripes and ecology evolve in a correlated fashion, have similar degrees of homoplasy and similar rates of character reversal, as measured by consistency and retention indices. The evolution of all three kinds of flank stripes, vertical bars, midlateral stripe and dorsolateral stripe, is associated with the evolution of ecological and behaviour traits, but only that of two out of eight kinds of head stripes: supraorbital and forehead stripe. Most head stripes have a higher degree of homoplasy, and one (LaS) has a lower rate of character reversal. On the other hand, the tendency to correlated evolution of particular head and flank stripes suggests that head and flank stripes with similar orientation respond in the same way to selection. Genetical/developmental constraints may be responsible for the complete associations between some melanin stripes. We interpret these results as evidence for ecology- and behaviour-driven convergent evolution of vertical and longitudinal stripe patterns, where the evolution of stripes on the head is less tied to ecology and behaviour than that on the flanks. Cross-head stripes are least, if at all, affected by ecology and behaviour. Being, nevertheless, often different between closely related species (Table 4, e.g. Voss, 1977, p. 71; Konings, 1988, pp. 112/113), they may play a role in species recognition, and the forces that shape their evolution may lay in the nonadaptive domain. Nevertheless, the results on stripes are at odds with the hypothesis of predominantly nonadaptive colour evolution.

What are the ecological and behaviour effects on stripe pattern evolution? Cichlids living in rocky habitats or among vegetation tend to be vertically barred. Rocky habitats and macrophyte beds are spatially and optically more structured than sand bottoms, open waters and even many river habitats. Background matching as a means of predator avoidance (Endler, 1978) could, hence, be one explanation for the association between habitat and vertical bars on the flanks. However, habitat and behaviour are significantly associated, and there could be an indirect effect of habitat on stripe pattern mediated by habitat effects on behaviour. Inhabitants of spatially structured littoral habitats are significantly less likely to evolve shoaling behaviour than inhabitants of open waters and sand bottoms, and shoaling affects stripe pattern evolution (see below). In structured littoral habitats the abundance of resources (*Aufwuchs*-dominated) usually varies on a smaller spatial scale than in open habitats but is temporally more stable than in offshore waters, where primary production is heavily dependent on seasonal upwelling of nutrient-rich deep waters (Lowe-McConnell, 1987). As a consequence, cichlids in structured habitats often show higher site-fidelity, stronger territoriality and less periodicity in sexual activity than cichlids in open habitats (Lowe-McConnell, 1979; Witte, 1981). Ethological studies on neotropical, West and East African cichlids have demonstrated motivational associations between aggressivity (territory

or clutch defence) and display of vertical bars (Nelissen, 1975; Voss, 1977; Baerends, 1984; Baerends *et al.*, 1986). Interhabitat differences in spatial and temporal variation of resource abundance, and their effects on prevalence of different motivational states in their inhabitant cichlid species therefore offer an explanation for the association between the evolution of habitat affiliation and vertical bars that is an alternative to background matching. Our data do not allow us to discriminate between the two hypotheses because we do not have enough detailed data on behaviour and, at the level of our analysis, the hypotheses yield identical predictions.

Cichlids that prey on evasive prey (mostly other cichlids) and shoaling cichlids tend to have a longitudinal stripe running along midbody. Diet and shoaling have independent and noncorrelated effects on this midlateral stripe. From ethological studies on neotropical and West African riverine cichlids, a motivational association between display of a midlateral stripe and social inferiority is known (Voss, 1977; Baerends, 1984; Baerends *et al.*, 1986). Hence, the presence of a midlateral stripe probably supports shoal cohesion by reducing aggressivity. It is less apparent what causes the effect of diet on this stripe. The stripe may have an appeasing effect on the prey fish. Alternatively it may interfere with visual perception of the predator by the prey. Experiments by Karplus *et al.* (1982) on the recognition of predator's faces by prey fish during frontal attack demonstrated that a disruptive pattern of dark patches, applied to the predator, reduced the aversive response of the prey fish (a pomacentrid). A midlateral stripe may have such effects because it disrupts the outline of the predator when viewed frontally.

4 The origin of male nuptial coloration is associated with the evolution of promiscuity, and in promiscuous cichlids the hue of male nuptial coloration changes across speciation events more often than any other trait. Taken together, this suggests that male nuptial coloration in East African cichlids does not only evolve rapidly under sexual selection by female mate choice (Deutsch, 1997; Seehausen *et al.*, 1997), but has arisen under female mate choice in the first place. There is very little diversity in hue among pair bonding and harem-forming species (compare photographs in Konings, 1988 with those in Konings, 1995 and Seehausen, 1996). Our data provide no evidence that stripe pattern is under sexual selection by female mate choice. Stripe pattern change was less common in the more strongly sexually selected (promiscuous) species pairs and the evolution of the mating system was not associated with that of particular stripes. We see the latter relationships between mating system and frequencies of change in coloration as suggestive but by no means conclusive because mating system is phylogenetically clustered (Table 4, Fig. 2). Therefore, we cannot dispense with the possibility that other variables, which map onto taxa in the same way as mating system, could also be responsible. Taken that such variables do not exist, we can think of two alternative but

nonexclusive hypotheses, each of which might explain the difference between melanic stripe pattern and nuptial coloration in respect to sexual selection. One would be that stripe patterns do not lend themselves so well to be moulded by sexual selection either because of their genetics, or because of their attributes as signals, or both. Crossing experiments indicate that the presence/absence of melanic stripes is determined by one or very few loci (Crapon de Caprona & Fritsch, 1984; authors unpublished data), which would make them unsuitable for runaway selection. Nevertheless, also such traits could flip up and down more quickly when they are under sexual selection that varies in strength or direction (Seehausen *et al.*, in press), and whether nuptial coloration is polygenic remains to be tested.

An alternative hypothesis is that ecological and behaviour demands on melanic flank markings, and possible genetic/developmental associations between certain stripes (that may have evolved as a consequence of ecological demands) constrain the possibilities of nonadaptive divergent evolution under sexual selection. Stripe patterns of cichlids develop early in the ontogeny and change with changing life style in the course of ontogeny (e.g. Voss, 1977), which is an independent line of evidence for ecological effects on stripe patterns. Guarded, and therefore gregarious cichlid fry often display a midlateral stripe that is replaced by other patterns when subadults give up the gregarious life style. In contrast, nuptial coloration formed by two other chromatophore systems develops only upon maturation, usually only in males, and is in many species switched off outside the breeding season. This relaxes the effects of natural selection and allows otherwise maladaptive response to sexual selection.

That pair bonding/harem-forming sister species differ more frequently in stripe pattern than promiscuous sister species is consistent with the hypothesis of rapid speciation of promiscuous cichlids under sexual selection on nuptial coloration, with which stripe pattern evolution does not keep pace. Alternatively, it could imply that stripe pattern evolves in the less sexually selected groups faster than in the more sexually selected groups. Although we cannot exclude this, the first explanation is much more likely. It is known that fishes are more variable in coloration where coloration is under intraspecific sexual selection (Endler, 1980; Seehausen *et al.*, 1997), variation which can lead to speciation if coloration is important in species recognition. Thus, where mating systems are conducive of sexual selection, nonadaptive speciation would become an abundant process, inflating the speciation rate.

This is consistent with what is known about speciation among the promiscuous Lake Malawi and Lake Victoria cichlids, where speciation is usually associated with changes in nuptial hue without changes in stripe pattern (Marsh *et al.*, 1981; McKaye *et al.*, 1982, 1984; Hoogerhoud *et al.*, 1983; Seehausen, 1996, 1997; Seehausen

et al., 1997, 1998). The same seems to apply for the Ectodini of Lake Tanganyika. Very similar to many haplochromines of Lakes Malawi and Victoria, the Ectodini are promiscuous female mouthbrooders and their colourful males often lek (Karino, 1997; Rossiter & Yamagishi, 1997). Sister species pairs from our trees often comprise a more yellow and a more blue one (*melanogenys-ochrogenys*, *dewindti-nasuta*, *perspicax-longiventralis*, *ventralis-schoutedeni*). Sister species pairs in Lake Victoria often comprise a more yellow to red and a more blue species too (Seehausen *et al.*, 1997), and McElroy *et al.* (1991) and Deutsch (1997) found predominantly yellow and blue among the rock-dwelling haplochromines of Lake Malawi. This may indicate that a similar model of speciation by sexual selection applies to promiscuous cichlids with male nuptial coloration in all three lakes.

Conclusions

Cichlids are known for fast evolutionary responses to changing ecological demands (summarized by Galis & Metz, 1998). Our analysis demonstrates that the evolution of melanic stripe pattern is historically just as little constrained as is ecological adaptation, and suggests that the diversity of stripe patterns found in lacustrine cichlid species flocks came about as a result of their radiation into a variety of ecological niches. This weakness of historical constraints on stripe pattern requires explanation. The rapid ecological radiation and extensive anatomical homoplasy in cichlids have been explained by a versatile anatomy that allows flexible feeding behaviour and phenotypic responses to new selection pressures, which can consequently be assimilated genetically (Liem, 1973; Greenwood, 1981; Galis & Drucker, 1996; Galis & Metz, 1998). We suggest that the weak historical constraints on melanic stripe pattern in the East African cichlid radiations is explained in a comparable way, namely by unusual chromomotor flexibility in the founder stocks.

The dark stripes on the body of cichlids are formed by large melanophores that are located in two layers in the skin (Baerends & Baerends-van Roon, 1950), and can, particularly in many riverine cichlids, very quickly be expanded and contracted under nervous control. Voss (1977), studying colour patterns of 20 African riverine cichlid species, found an exceptionally rich 'optical language' in many species. Species of the widely distributed genus *Tilapia* and others were able to switch on and off distinct vertical and longitudinal flank stripes, as well as several kinds of head stripes and could, by doing so, create several stripe combinations, each of which was associated with particular motivational states, seemingly serving intraspecific signalling of motivation. Baerends (1984) found similar optical language in a neotropical (*Aequidens*) and another West African (*Chromidotilapia*) riverine cichlid, but Voss (1977) observed less flexible

chromomotor patterns in East African riverine species (*Oreochromis*, haplochromines). The latter are promiscuous and have relatively simple courtship behaviour, while the other African species studied (and Baerends' neotropical species) are monogamous with lasting pair-bond and complex courtship behaviour. From this, Voss concluded that chromomotor flexibility had evolved to assist intraspecific communication during reproduction, and was lost when reproductive behaviour became simplified. Now we know that the reality is yet more complicated. Relatively inflexible chromomotor patterns are characteristic not only for East African promiscuous river cichlids but for most endemic East African lake cichlids with diverse mating systems. A good example are the lamprologines, monogamous and harem-building species with complex social interactions (Taborsky & Limberger, 1981; Taborsky, 1984; Kuwamura, 1997; Nagoshi & Yanagisawa, 1997) but strongly reduced chromomotor flexibility. While species such as *Neolamprologus cylindricus*, *N. compressiceps* and *N. calvus* exhibit vertical bars continuously, others such as *Julidochromis* spp., *Chalinochromis* spp., *Telmatochromis vittatus* and *T. bifrenatus* continuously exhibit longitudinal stripes, and again others such as *N. leleupi* are continuously plain. Similar examples could be drawn from the species flocks of Lakes Malawi and Victoria. Different from Voss, Baerends *et al.* (1986) proposed the hypothesis that cichlid stripe patterns have primarily evolved to serve predator avoidance and possibly predator intimidation, and that adaptation to the signalling of motivation has taken place secondarily superimposed on the primary patterns.

All recent phylogenetic estimates for the family Cichlidae (Oliver, 1984; Stiassny, 1991; Lippitsch, 1995; Suetmann *et al.*, 1995; Zardoya *et al.*, 1996; Streelman & Karl, 1997), regardless of the type of character and the method of reconstruction used, suggest that the major melanin stripes (vertical bars, midlateral stripe and orbital stripes), as well as chromomotor flexibility, had evolved in riverine (and estuarine) environments within the family before the East African lacustrine (Tanganyikan) primary radiation took place. Flexible chromomotor patterns may be the plesiomorphic condition in this primary radiation (Fig. 3). The basal Tanganyikan genus *Boulengerochromis*, many members of the internally not yet resolved sister groups of the Tanganyikan radiation, as well as many neotropical cichlids possess a very flexible pattern. These phylogenetic estimates further suggest that the origin of intraspecific pattern complexity (combination of different kinds of stripes) and flexibility coincided with the evolution of large interspecific diversity in melanin pattern (Fig. 3). Parts of the East African lacustrine radiation are unique in combining little intraspecific flexibility with large interspecific diversity. Under whatever kind of selection it arose in the first place, melanophore chromomotor flexibility, once present,

will inevitably come under all selection forces that operate on coloration. It allows instantaneous phenotypic response not only to short-term predator avoidance and communication demands, but to longer term demands on coloration posed by changing ecological conditions too. Such phenotypic response could consequently be assimilated genetically. Selection, acting upon the nervous control of melanophores, could emphasize, or fix in switched-on or switched-off state, particular elements of a previously flexible stripe pattern, possibly achieved by changes in regulatory gene interactions. It has been demonstrated in butterflies that effects of regulatory gene interactions on phenotypically plastic colour patterns can make the latter radiate rapidly (Brakefield *et al.*, 1996).

When riverine cichlids first colonized Lake Tanganyika they came from seasonal and microspatially variable riverine environments into more stable, quasi-oceanic environments (Lowe-McConnell, 1987). In the course of the ecological radiation that followed, in which populations invaded different niches with different average demands on coloration, chromomotor flexibility for different parts of the stripe pattern may have been lost in different niches. Under this model, stripe pattern evolution in the East African cichlid radiation would have to be understood as evolution of regulatory gene interactions that modify the nervous control over parts of already existing chromatophore systems. Despite the loss of phenotypic plasticity during stripe pattern radiation, genetic variation at the colour loci could be retained. This would explain the observed explosive diversification of stripe patterns, the high degree of correlated homoplasy and character reversal in stripe pattern and ecology, and the reduced chromomotor flexibility in lacustrine cichlids. Forthcoming studies can test this hypothesis by determining, with comparative experimental approaches, the temporal sequence and functional correlates of the major events in cichlid stripe pattern evolution, and their genetical basis.

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Appendix A

Sources of coloration data

(only first author and year are given of sources that are in the reference list)

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