Divergent selection during speciation of Lake Malawi cichlid fishes inferred from parallel radiations in nuptial coloration

Charlotte J. Allender[†‡§], Ole Seehausen[†§¶‖], Mairi E. Knight[†], George F. Turner[¶], and Norman Maclean[†]

[†]Biodiversity and Ecology Division, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, United Kingdom; and [¶]Molecular and Evolutionary Ecology Group, Department of Biological Sciences, University of Hull, Hull HU6 7RX, United Kingdom

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Repeated evolution of the same phenotypic difference during independent episodes of speciation is strong evidence for selection during speciation. More than 1,000 species of cichlids, >10% of the world's freshwater fish species, have arisen within the past million years in Lakes Malawi and Victoria in eastern Africa. Many pairs of closely related sympatric species differ in their nuptial coloration in very similar ways. Nuptial coloration is important in their mate choice, and speciation by sexual selection on genetically or ecologically constrained variation in nuptial coloration had been proposed, which would repeatedly produce similar nuptial types in different populations, a prediction that was difficult to test in the absence of population-level phylogenies. We measured genetic similarity between individuals within and between populations, species, and lake regions by typing 59 individuals at >2,000 polymorphic genetic loci. From these data, we reconstructed, to our knowledge, the first larger species level phylogeny for the most diverse group of Lake Malawi cichlids. We used the genetic and phylogenetic data to test the divergent selection scenario against colonization, character displacement, and hybridization scenarios that could also explain diverse communities. Diversity has arisen by replicated radiations into the same color types, resulting in phenotypically very different, yet closely related, species within and phenotypically highly similar yet unrelated sets of species between regions, which is consistent with divergent selection during speciation and is inconsistent with colonization and character displacement models.

Repeated differentiation into the same pairs of ecological or mating phenotypes during independent episodes of speciation is strong evidence for speciation by selection (1–3). Because species continue to diverge in other traits after speciation, such pairs are most readily detected where many species have recently diverged from few ancestors, as in geologically young adaptive radiations (4). The haplochromine cichlids in Lakes Victoria and Malawi in eastern Africa (5) form the largest recent adaptive radiations of vertebrates and account for >10% of the world's freshwater fish species (6–8). Parallel origins of similar morphologies in different lakes has been documented (9), but closely related species within each lake generally have similar morphologies (10), but differ in nuptial color patterns, often in similar ways (11–13). Nuptial coloration is important in their mate choice (13, 14) and speciation by sexual selection on genetically or ecologically constrained variation in nuptial coloration had been proposed to explain its rapid evolution (15, 16). Other candidate explanations include genetic drift in small populations and reproductive character displacement in secondary sympatry.

Even in the stenotopic rock-dwelling Mbuna group, populations rarely show the genetic imprints of bottlenecks (6, 17), and the number of migrants between populations is typically too large for divergence by drift (17–19). Many haplochromines are sexually dimorphic in color, and those that radiated rapidly possess a polygynous mating system. Speciation by runaway sexual selection (20–24) could lead to large diversity of color patterns, but most color variation among the rapidly radiated Lake Victoria cichlids and Lake Malawi Mbuna appears highly repetitive and can be partitioned into a small number of core patterns that are similar between the lakes (ref. 25 and Fig. 1*a*). Sympatric and parapatric species with similar morphologies usually have different color patterns, and most regional assemblages are composed of several patterns. Differences in these patterns are typically associated with prezygotic reproductive isolation (14, 26–30).

These latter observations stimulated suggestions that divergent or disruptive sexual selection may cause speciation without requiring complete geographical isolation (15, 16, 22, 23, 31). However, rather than being closely related to each other, as that hypothesis predicts, geographically proximate species with different color patterns could be more closely related to geographically distant species with the same or with different color patterns. The first scenario is predicted if divergent selection on color pattern had not often caused speciation, and color pattern types, once evolved in one site, spread around the lake. The second scenario is predicted if secondary contact, established during range expansion of allopatrically evolved species, was accompanied by sexual character displacement (32).

Any of these scenarios could account for the wide and disjunct geographical distribution of color patterns and the coexistence of several in most local communities, but they make different and exclusive predictions for phylogenetic relationships and relationships between genetic and geographic distance. If color patterns evolved once, and subsequently spread around the lake, populations of similar color from different regions will be more closely related to each other than species of different color in the same region. We call this pattern the multiple colonization model (Fig. 1*c*). Alternatively, character displacement would predict reciprocal associations between transitions in geographical range and transitions in color between species pairs (Fig. 1*d*). Finally, frequent speciation with divergent or disruptive selection important in the initial stage predicts radiations into different color patterns within regions, which are replicated between regions (Fig. 1*e*).

Distinguishing between the scenarios and determining their prevalence in cichlid radiations is crucially important to unraveling the mechanisms of explosive speciation. It is only possible if speciation was sufficiently recent for range expansion within a single lake to not have erased the phylogeographic signature. Yet, it requires sufficient genetic differentiation for reconstruction of a species-level phylogeny. This combination of requirements has hitherto prevented attempts to use phylogenies to this end. Much of the Mbuna diversity is very recent (6), and earlier attempts to test speciation models were often inconclusive because of shared se-

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Abbreviations: AFLP, amplified fragment-length polymorphism; NW, Northwest; SE, Southeast.

[‡]Present address: Comparative Genomics and Genetics Group, Horticulture Research International, Wellesbourne, Warwick CV35 9EF, United Kingdom.

[§]C.J.A. and O.S. contributed equally to this work.

[‖]To whom correspondence should be addressed. E-mail: o.seehausen@hull.ac.uk.

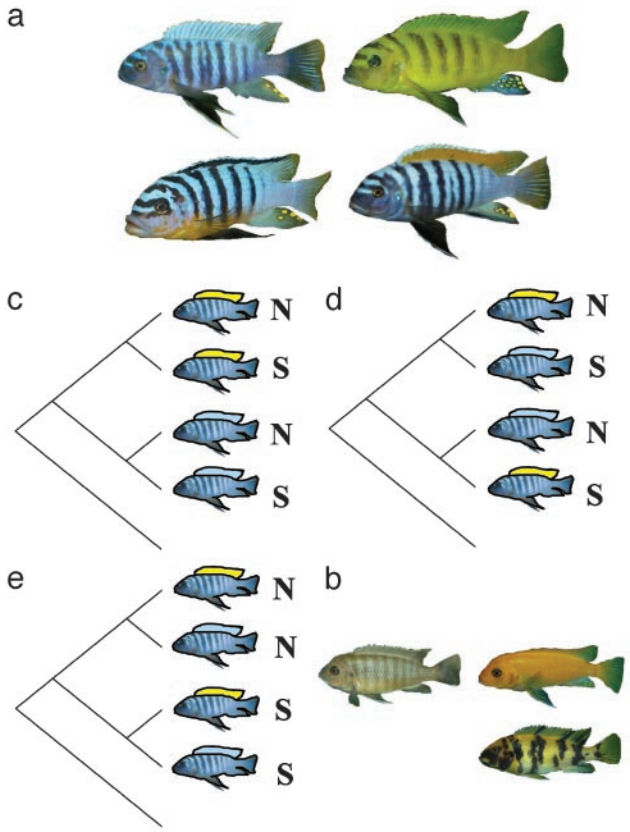

Fig. 1. The core color patterns observed among Mbuna cichlids and evolutionary models of radiation in color pattern. (*a*) Core male nuptial patterns: all blue, all yellow, yellow chest, and yellow/orange dorsal. (*b*) X-linked patterns: barred, orange, and blotched. (*c*) Colonization model (model A): colors evolved once, geographically proximate species are more closely related to geographically distant populations of the same color than to each other. Letters denote geographical regions. N, North; S, South. (*d*) Character displacement model (model B): colors evolved repeatedly, sister species occur in different geographical regions. (*e*) Divergent selection model (model C): colors evolved repeatedly, geographically proximate species are more closely related to each other than to geographically distant populations of the same or different color.

quence polymorphisms in mitochondrial genes (33, 34), inconclusive phylogenetic signal, or absence of between species phylogeographic signature due to focus on a single lake region within which species are now widely distributed (35). Recently strong evidence (36) for convergent origin of the same color pattern in two distant populations of the same species complex of Lake Malawi cichlids has been produced, to our knowledge, for the very first time. However, the authors of that paper caution that introgression between sympatric species could have produced the pattern in the particular case they studied.

In this article, we report a combination of approaches that overcome methodological limitations of previous approaches. We constructed a strongly supported phylogenetic tree from multilocus [amplified fragment-length polymorphisms (AFLPs)], genotypes for 20 taxa of Mbuna, from three different regions of Lake Malawi, including 14 taxa of the *Maylandia zebra* complex. We mapped the complete published distribution record of each species (Fig. 2*a* and refs. 37–40). Because within each region most species now have wide distribution ranges, our data cannot resolve the microgeography of speciation, but they are powerful to distinguish between multiregional radiation driven by local processes and diversification driven by larger-scale processes, such as cycles of crosscolonization between regions. Matrix association tests and parsimony analysis of evolutionary transitions in color pattern and geographic region provided us with tests of the prevalence of these alternative evolutionary scenarios in a haplochromine cichlid radiation.

Materials and Methods

Taxonomic Distribution of Color Patterns in Mbuna. More than 200 species of Mbuna are currently known (11). We viewed in total several thousand photographs of all species. Seven common color patterns are similar to those described earlier for Lake Victoria cichlids (25). Four have usually (but not always) male-limited expression, defined by the presence/absence and location on the body of yellow/orange coloration: "all blue" (no yellow/orange; body and dorsal fin blue); "yellow chest" (yellow variably extending upwards from the chest to head and flanks, otherwise blue); "orange dorsal" (body blue, dorsal fin yellow/orange) and "all yellow" (entire body yellow; Fig. 1*a*). Three other patterns occur commonly in females and rarely in both sexes: brown barred (*BB*), dark blotches on orange to white background (*OB* coloration), and bright orange (referred to as *O*, ranging from orange to yellow and white; Fig. 1*b*). Experimental studies suggest that variation between *BB* and *OB* is X-linked, and that *O* loci only affect coloration in the presence of *OB* genes (41, 42). To quantify frequency of occurrence and taxonomic distribution of the core patterns, we analyzed underwater photographs of 1,173 males of 293 species and 612 females of 254 species and geographic varieties of Mbuna (40). Because some species exhibited more than one pattern, we calculated for each pattern the number of taxa (species and geographic varieties) in which it was present.

Choice of Taxa. To build a phylogenetic tree that would allow us to test the three alternative speciation scenarios, we chose to focus on the *Maylandia zebra* species complex, which is possibly the most species-rich of all Mbuna species complexes. We sampled 14 populations representing all core color types each from two or three different localities on different sides of the lake. These included three populations of the widely distributed blue barred (*BB*) *M. zebra* [Northwest (NW), central East, and Southeast (SE)]. We also included three species of the closely related genus *Tropheops* and several other Mbuna, with *Rhamphochromis esox* (a Lake Malawi non-mbuna), four species of haplochromine cichlids from Lake Victoria, and the tilapiine cichlid *Oreochromis niloticus* as nested outgroup. Three individuals were sampled from most ingroup taxa (Table 1, which is published as supporting information on the PNAS web site, provides species, sampling sites, sample sizes, and color character states).

Molecular Methods. The original AFLP protocol (43) was followed, except that the restriction and ligation steps of the procedure were combined. The restriction enzymes used were *Eco*RI and *Mse*I. Primer sequences for preselective PCR were GACTGCGTACCAATTCA and GATGAGTCCTGAGTAAC. An additional two bases were added to the 3′ end for selective PCR. In total, 18 primer pairs were used (AC-CA; AC-CC, AT-GG, AT-GC, AA-CA, A-CT, AA-GC, AA-CC, TA-CA, TA-CT, TA-CC, AG-CT, AG-GC, AG-AC, TT-CA, TT-GG, TG-GG, TG-AC). Bands were visualized by using an ABI 377 sequencer and GENESCAN software (Applied Biosystems) with internal size standard (GS-500 ROX; Applied Biosystems). All samples amplified with one primer pair were separated on the same gel. We imported GENESCAN files into GENOGRAPHER (http://hordeum.oscs.montana.edu/genographer). Peaks between 75 and 490 bases could be scored unambiguously for presence/absence. Twelve samples were subjected to three different restriction-ligation reactions. Presence/absence of 87 bands (one primer pair) yielded a reproducibility of 92.4 ± 3%. In total, 2,189 polymorphic AFLP sites (122 ± 27.4 per primer pair) were obtained.

Phylogeny Reconstruction. Adequacy of phylogenetic signal was tested by using PAUP* VB4.01 (44) by plotting the length distribution

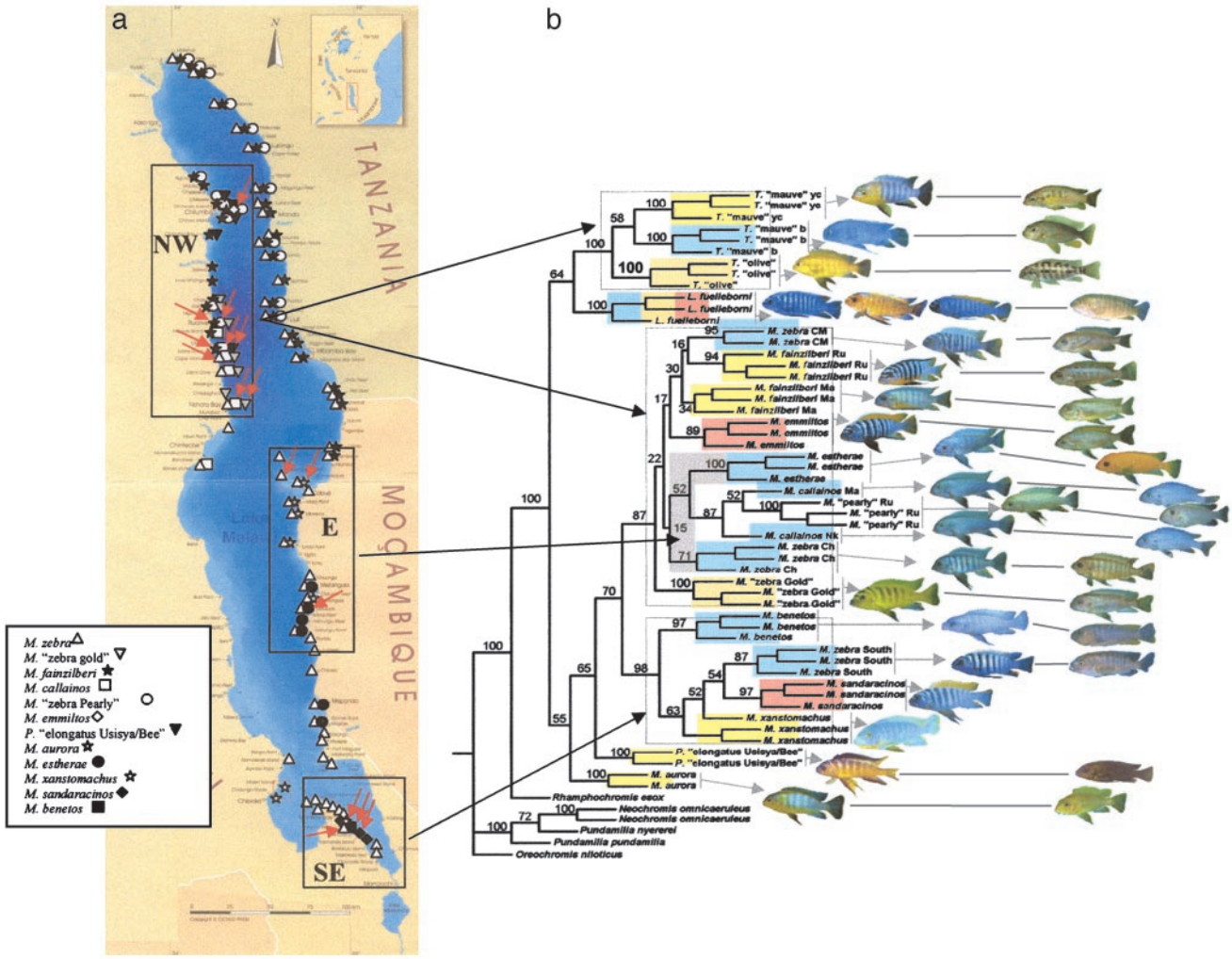

Fig. 2. (*a*) All published distribution records of the *Maylandia*/*Pseudotropheus* species in our phylogeny (data compiled from refs. 11 and 37–40). Our sampling localities are indicated by red arrows. White asterisks in the east region represent *M. aurora*, and white asterisks in the SE region and west of it represent *M. xanstomachus*. (*b*) Phylogenetic tree for the *Maylandia zebra* species complex and several other Mbuna (*M., Maylandia*; *T., Tropheops*; *L, Labeotropheus*; and *P., Pseudotropheus*), with *Rhamphochromis esox* (a Lake Malawi non-mbuna), four species of haplochromine cichlids from Lake Victoria, and the tilapiine cichlid *Oreochromis niloticus* as nested outgroup. See *Materials and Methods* for tree construction. Colored blocks unite individuals of the same population and indicate the male nuptial coloration of populations: blue, all blue; yellow, yellow chest; orange, all yellow; red, orange dorsal. Open boxes and gray box connected by arrows to boxes on the map indicate geographical regions. *Labeotropheus fuelleborni* is a complex of geographically differentiated populations that can exhibit any of the color patterns. *Pseudotropheus elongatus* Usisya represents a complex of species that can also exhibit several color patterns. Images of males (*Left*) and females (*Right*) of the same taxon are connected by a gray line. Females of *M. sandaracinos* and *M. xanstomachus* resemble those of *zebra* South (photographs of these were not available). Photographs of *Tropheops* species, *Labeotropheus* species, *M. benetos*, *M. zebra* South, *M. sandaracinos*, and *P. elongatus* courtesy of A. Konings (Cichlid Press, El Paso, TX). Photograph of *M. xanstomachus* courtesy of A. J. Ribbink (South African Institute for Aquatic Biodiversity, Grahamstown, South Africa).

of 100,000 random trees. The g1 statistic (45) revealed significant nonrandom structure in the entire data set (g1 −1.499; 64 samples; 2,189 variable sites), and among the Mbuna (g1 −0.39; 56 samples). Pairwise genetic distances were calculated with the restriction-site program RESTDIST (ref. 46; accessions with incomplete genotypes omitted) and trees were constructed with the Fitch and Margoliash algorithm with unconstrained branch length [ref. 47; implemented in FITCH in PHYLIP3.6A2 (46)]. Each of 100 bootstrapped data sets was analyzed 10 times with different random input orders, and with local and global optimization.

Parsimony Analysis of Color Evolution and Geographical Range. Transitions in color pattern were reconstructed by using parsimony in MACCLADE 3.05 (48). We used an unordered, as well as an ordered, transformation type: blue with yellow chest = 1, all blue = 2, and blue with orange dorsal = 3. All yellow was coded 0 (one step from yellow chest) and 4 (one step from orange dorsal) in separate analyses. Female coloration was treated as four ordered states: barred (*BB*) = 0, *BB*/*OB* polymorphism = 1, *BB*/*OB*/*O* = 2, and *OB*/*O* = 3. Outgroups were removed as not to influence color reconstruction in the Mbuna. To distinguish between character displacement (model B) and divergent selection during speciation (model C), we reconstructed the history of changes in geographical distribution in the *zebra* complex, subjecting the three lake regions (Fig. 2*a*) to a parsimony analysis (states ordered as NW-central East-SE). Changes in color pattern were then mapped onto region trees.

Speciation Model Tests. Provided range expansion with secondary contact has not erased the phylogeographic signal, *in situ* divergence (defined as divergence within one region) predicts that geographically proximate species are genetically more similar to each other than are geographically distant species, whereas multiple colonization (with or without character displacement) makes the opposite prediction.

We used Mantel randomizations (49) to examine the relationship between genetic and geographical distances within the *M. zebra* species complex and the match with predictions of alternative speciation models (Fig. 1 *c–e*). For the model tests, we calculated associations between the matrix of observed genetic distances and design matrices: model A (multiple colonization without character displacement): species of same color plus different region more similar ($D = 0$) than those of different color ($D = 1$); model B (multiple colonization with character displacement): species of different color plus different region more similar ($D = 0$) than species of same color or region ($D = 1$); model C (*in situ* divergence): species from same region more similar ($D = 0$) than species from different regions ($D = 1$). Furthermore, we used two colonization matrices without constraints on color: model D (allopatric speciation on full geographical scale): species from different regions more similar than species from same region; model E (allopatric speciation on intermediate geographical scale): species from the NW or the SE more similar to species from the central East coast ($D = 0$) than to each other or to species from the same region ($D = 1$).

Multiple colonization of a region and subsequent persistence with gene flow predicts genetic similarity between sympatric species too, but, contrary to other models, predicts the largest genetic distances to occur between species at intermediate geographical distance, e.g., between sites within the same region (historically unrelated species without opportunity for hybridization). To test whether genetic similarity among geographically proximate species was caused by current gene flow, we modified matrices of colonization models (A, B, D, and E) to incorporate effects of hybridization among sympatric, parapatric, and microallopatric populations by changing their distances in the matrix from 1 to 0. We tested this hybridization effect for a range of geographical proximities ($\leq$10, $\leq$20, and $\leq$30 km) and for a range of relative strength of the hybridization and ancestry effects ($D = 0/D = 0$, $D = 0/D = 0.5$, and $D = 0.5/D = 0$). All Mantel randomizations were calculated in MANTEL.XLA V1.2 (50).

Test of Selection on Coloration. The species on our tree are fixed for all of the color patterns that differentiate them (11, 40), except that seven populations are polymorphic for X-linked color. As a first crude approximation to the probability that fixation of color patterns happened by chance, we modified a gene-tree-based method for comparing phenotypic and molecular genetic fixation rates (24) for use with multilocus genetic information. We calculated, for each of 500 AFLP loci that were variable among species in the *zebra* complex, the ratio of number of populations fixed for the nonancestral allele over number of polymorphic populations. The derived allele was almost always the band presence allele. To correct for effects of reproducibility of AFLP bands (92%), we multiplied the number of polymorphic populations for each locus by 0.92. By sampling three individuals per population for determining variation at a dominant molecular marker, we underestimate the number of populations that possess the derived allele, and overestimate the fraction fixed for it. The ratio is the per-locus probability that a derived allele, after its frequency has risen to $\approx$15% or more (to be represented at least in one copy in one of three individuals), increases to close enough to fixation to be present in at least one copy in each of three individuals in a sample.

The ratio, therefore, is a high-end approximation of the probability that a neutral allele, once in a population, becomes fixed by chance. It can, however, only serve as a crude guideline to whether the derived color patterns, fixed in species on our tree, may have become fixed by chance, because it is unknown how mutation rates affect fixation probabilities at AFLP and at color loci. It is likely for both types of loci, that recurrent mutation is elevating fixation rates for absence and suppressing fixation rates for presence of characters. Assuming that mutation does not drive the fixation of AFLP loci more than the fixation of color patterns, we cautiously suggest that when fixation of color pattern by drift is the null hypothesis, the AFLP-derived estimate of neutral fixation probabilities is conservative because: (*i*) the initial frequency of novel color alleles is most likely $<$15%; (*ii*) because it is likely that not all AFLP loci are neutral, and, finally; (*iii*) because color patterns may often be determined by more than one gene, in which case the probabilities for fixation would compound.

Second, we calculated reciprocal fixation probabilities for pairs of geographically proximate species that are sister taxa on our phylogeny, as the fraction of AFLP loci reciprocally fixed for presence and absence alleles among all loci that are variable in the pair. Correction for effects of 92.4% reproducibility was done by removing 7.6% of singletons (AFLP bands uniquely present or absent in just one individual).

Results and Discussion

Repetitiveness of Color Patterns. Ninety percent of all Mbuna species possess at least one of four male nuptial patterns and 82% possess at least one of three X-linked patterns in females. All blue appeared in males of 120 of 293 taxa (41%), yellow chest in 90 (31%), all yellow in 48 (16%), and orange dorsal in 30 (10%). Only 26 taxa (9%) did not exhibit any of the four male patterns. Each genus with more than one species contained several of the male patterns. Barred females appeared in 183 species (72%), *OB* in 15 (7%), *O* in 8 (3%), and bright yellow or white similar (and possibly homologous) to *O* in 36 (14%). Only 10 taxa (4%) did not exhibit any of these female patterns.

The Phylogeny. In overlapping sections, the phylogenetic tree (Fig. 2*b*) is completely consistent with previously published AFLP-based phylogenies for haplochromine cichlids (10, 51). Within the Mbuna, the genera *Tropheops*, *Labeotropheus*, and *Maylandia*/*Pseudotropheus* form three clades. *Metriaclima aurora* and *Pseudotropheus elongatus* Usisya are basal species within the latter. The rest of this clade is henceforth referred to as the *zebra* complex. All but one population emerged monophyletic with bootstrap support ranging from 34% to 100% (mean 92.4 $\pm$ 16.3%, $n = 18$ populations), indicating that we sampled a sufficient number of loci to overcome phylogenetic effects of incomplete lineage sorting (34), even among the very closely related species and populations of the *zebra* complex. The only exception was *Metriaclima xanstomachus*, which emerged as paraphyletic with regard to one sympatric and one parapatric species.

The *M. zebra* complex is deeply divided into an SE and an NW/central East clade. Most populations are most closely related to geographically proximate populations of different core color pattern. For instance, each of the three populations of the lake-wide occurring all blue *M. zebra* (commonly referred to as *BB* zebra) is more closely related to geographically proximate species of another color pattern [*fainzilberi* (yellow chest), *estherae* (*O*/*OB*-morph females), and *xanstomachus* (yellow chest), respectively] than to what were thought to be conspecific *BB zebra* populations from elsewhere in the lake.

Single Versus Repeated Origin of Color Patterns. Each of the four male nuptial and three X-linked color patterns arose or was lost repeatedly within the *zebra* complex and additional times between the other Mbuna (see Fig. 3 and Table 2, which are published as supporting information on the PNAS web site). Extreme evolutionary lability of male color pattern is reflected in unusually low retention indices (Table 2). In fact, the number of transitions between color patterns required on our tree is very close to the maximum possible on any tree. Ambiguities with regard to direction of transitions only affected all blue versus yellow chest (Fig. 3*a*) and presence or absence of *OB* (Fig. 3*b*). If internal branches with conflict are resolved as yellow chest, the all blue species *M. zebra BB* must have evolved three times independently, once in each of the three lake regions. Entirely blue or yellow species would have

evolved from a blue ancestor with yellow chest. Alternatively, if the internal branches are resolved as all blue, *M. zebra BB* is the most parsimonious paraphyletic ancestor of many *zebra* species with other color. Yellow chest would then have evolved within the *zebra* complex three times in parallel and was acquired up to five times and lost again at least once among all Mbuna species on our tree. All yellow and orange dorsal must each have evolved in parallel in each species with these colors on our tree.

OB/*O* female color originated three to five times among the sampled species (two to four times within the *zebra* complex) and may have been lost twice within the *zebra* complex (Fig. 3*b*). Almost fixation of the orange morph in females in the Minos Reef population of *Maylandia estherae* (most females are orange, but a few are *OB*) and complete fixation in *Maylandia* "zebra pearly" (where the amelanic orange females are actually white) appear to have happened independently.

Regional Origin Versus Multiple Colonization With Character Displacement With or Without Hybridization. Between 85% and 100% of male nuptial color pattern changes occurred within, as opposed to maximally 15% coinciding with switches between regions. Between 57% and 86% of transitions in female coloration also occurred within regions (Fig. 3*c* and Table 3, which is published as supporting information on the PNAS web site). Pairwise genetic distances were significantly smaller between individuals of the same population than between individuals of different populations within the *zebra* complex [*t* test assuming unequal variances(calculated in SPSS 11.0), $n_1 = 35, n_2 = 38, t = -5.07, P < 0.0001$, each individual represented once by its mean value]. Hence, it was justified to use population distances (the mean distance between 2×3 individuals) in Mantel tests of models of population divergence. Conservatively including only one in any group of geographically adjacent putatively conspecific populations, we found that genetic distance between species within the *zebra* complex was significantly positively associated with geographic distance (standardized Mantel correlation $r = +0.36$, $P = 0.007$), as expected if population divergence occurred within regions as opposed to between regions followed by multiple colonization of regions and reproductive character displacement. This finding does not rule out character displacement as an ingredient to speciation within a region.

Of the different speciation models, only predictions from model C and E significantly matched the data (standardized Mantel correlations: A, $r = -0.06$, $P = 0.81$; A with hybridization, $r = +0.01$, $P \geq 0.45$; B, $r = -0.20$, $P = 0.93$; B with hybridization, $r = -0.20$, $P \geq 0.83$; C, $r = +0.39$, $P = 0.01$; D, $r = -0.32$, $P = 0.97$; D with hybridization, $r = -0.20$, $P \geq 0.45$; E, $r = +0.22$, $P = 0.01$; and E with hybridization, $r = +0.12$, $P \geq 0.02$). A stepwise multiple matrix regression revealed that models C and E explained mostly complementary fractions (model C a larger fraction than model E) of the variance in genetic distances ($r = +0.55$ for the combined model). Residual variance in genetic distances was not associated with geographic distance (standardized Mantel correlation: $r = -0.01$, $P = 0.39$) which is contrary to expectations of recent hybridization among sympatric species.

Selection on Color. The average ratio of populations fixed for a derived AFLP allele over polymorphic populations was 0.080 ± 0.018 (calculated for five nonoverlapping sets of each 100 polymorphic AFLP loci). Hence, the probability of chance fixation in a population for a novel allele starting from a frequency of ≈15% or higher is estimated at ≤0.08. Given that novel color patterns probably start out at lower frequencies, the probability that derived male color patterns would have become fixed by chance in all six (if all blue was ancestral) or seven (if blue with yellow chest was ancestral) species with these colors that were sampled does therefore seem small. Seven of 14 *zebra* populations were polymorphic for X-linked color, five were fixed for *BB*, and two for *OB*/*O* coloration. The difference between *BB* and *O* is determined by at least two but probably more genes (41, 42). Hence, the probability that one in nine sampled populations that have *OB* became fixed by chance is ≤0.054. The probability that a second population became almost fixed for the same trait by chance is even smaller.

Reciprocal fixation probabilities among the three most closely related species in the SE region were: $P \leq 0.002$ for *M. xanstomachus* and *Maylandia sandaracinos* that occur sympatrically and differ in two fixed color traits (Table 1), $P \leq 0.047$ for *M. xanstomachus* and *M. zebra* South, and $P \leq 0.019$ for *M. sandaracinos* and *M. zebra* South that occur parapatrically and differ in one fixed color trait. The orange versus blue dorsal fin is probably determined by more than one gene (52), which would further reduce these fixation probabilities. We also calculated divergent fixation probabilities for the most closely related species pair in the NW region: *Maylandia callainos* and *M.* "zebra pearly" have a microallopatric distribution, separated by ≈7 km (Mbowe Island to Ruarwe) and differ in one reciprocally fixed phenotypic trait. Their reciprocal fixation probability at AFLP loci is ≤0.048.

Parallel Radiations in Nuptial Coloration. We conclude that each derived color pattern has been acquired repeatedly in parallel between and within genera. If the taxa sampled for our tree are representative, the Mbuna radiation is associated with >250 transitions between four male and three female core color patterns. Most species appear to have arisen from geographical neighbors of different color. We interpret the absence of an association between genetic similarity and geographic distance on a sympatric to microallopatric scale, despite the highly significant association on the larger geographical scale, as evidence for that gene flow among species has ceased, and that the genetic similarity between geographically proximate species is not explained by current gene flow. This result does not rule out hybridization in the past. Our data are consistent with several speciation models that involve past hybridization, such as local radiation from a hybrid population derived from migrants of several older species, or speciation by reinforcement of reproductive isolation between several colonizing and hybridizing populations. Radiation from a hybrid population differs from the other scenarios in that the genetic basis for each color pattern may have had to evolve just once, but both scenarios have in common with radiation from a single colonizing species (model C) that reproductive isolation has most likely evolved *in situ* by divergent or disruptive selection (sexual or natural selection or a combination of both). That fixation rates of color patterns in sister species appear to significantly exceed those of neutral genes, is consistent with this conclusion.

Because within each region most species now have wide (overlapping or mosaic) distribution ranges, the most probable microgeography of speciation will have to be inferred from current potential for isolation between habitat patches within regions. Population genetic estimates of number of migrants between NW coast populations suggest very little gene flow between habitat patches, implicating potential for microallopatric speciation under divergent selection (53). On the other hand, distribution patterns and population genetic estimates of gene flow between islands in the SE arm are more consistent with divergence with gene flow and disruptive selection (17). Either way, selection on nuptial patterns resulted in parallel origin of phenotypically highly similar, yet unrelated sets of species in different regions of the lake and phenotypically highly divergent, yet closely related species within regions.

Possible Causes of Determinism in the Radiation of Nuptial Color Patterns. The degree of repetitiveness in color pattern in the parallel radiations of Mbuna cichlids suggests ecological and/or genetic constraints. Sexually selected color signals are most likely constrained by visual ecology. The clear waters of Lake Malawi transmit blue light most effectively. Mbuna cichlids possess visual pigments with peak sensitivities on UV, blue, green, and yellow

(54). If visual conspicuousness determines mating success, and if conspicuousness is determined by reflectivity, sexual selection should favor blue. However, if contrast determines conspicuousness, yellow, which is maximally offset from the ambient light, would be favored (55, 56). The simultaneous action of both mechanisms, either in different microhabitats or individuals with different visual preferences, might be a source of disruptive selection and explain the predominance of blue and yellow.

Observations on associations between nuptial pattern and ecology among Lake Victoria cichlids indicate that the orange dorsal phenotype tends to be associated with a planktivorous feeding style, whereas yellow (or red) chest is often associated with a benthivorous feeding style (57). In planktivorous species, females typically forage in the water column above the males that defend territories on the lake bottom, whereas in benthivorous species, females and territorial males forage on similar levels in the water column. Hence, the effectiveness of different positions of nuptial signals on the male body would vary with feeding ecology, which may exert selection on male nuptial patterns. Further ecological and physiological studies are required to test these hypotheses.

Testing Speciation Models in Explosive Radiations. Reconstruction of phylogenies among the shortest branches in adaptive radiations has been a major stumbling block to testing much debated models of speciation among explosively radiated groups. These groups have often been referred to as star bursts, implying they are phylogenetically unresolvable. However, even in explosive radiations, population divergence and speciation is likely to be to some extend sequentially structured. This is true, even if reticulate evolution has been common. It is essential to recover this structure to discriminate between evolutionary processes before, during, and after speciation as is required to test models of speciation in adaptive radiation. Scoring AFLPs at a sufficiently large number of loci allowed us to reconstruct population-level phylogenetic relationships within the most rapidly evolved cichlid species complex in Lake Malawi. We observed a linear relationship between tree support [mean bootstrap support over all nodes (y)] and number of loci scored (x) with no indication for asymptotic flattening ($y = 0.0102x + 43.613$; $R^2 = 0.94$; $P < 0.001$; as compared with $R^2 = 0.94$ also for quadratic and power models, and $R^2 = 0.91$ for a logarithmic model). This finding suggests that after scoring ≥2,000 variable loci, we have not exhausted the potential to recover further phylogenetic signal. Hence, the method holds considerable promise in radiations that have previously been phylogenetically intractable (10, 51, 58, 59).

Explosive Speciation and Adaptive Radiation in African Cichlid Fishes. A recent quantitative genetics investigation into functional morphology of Lake Malawi cichlids (60) demonstrated that strong directional selection on the shape of the feeding apparatus is likely to be responsible for the diversity of functional morphologies between genera of Lake Malawi cichlids. Here, we demonstrated that divergent or disruptive selection on coloration most likely explains the diversity of color patterns among species within genera. Simultaneous or alternating action of these selection forces may explain the explosive radiations of cichlids of the haplochromine group. Research into the functional ecology, genetics, and development of color patterns and color vision, and into the role of hybridization, is now required to characterize the interplay between selection, gene flow, genetic, and developmental properties that may hold the key to understanding the high rates of speciation in this adaptive radiation.

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1. Schluter, D. & Nagel, M. (1995) *Am. Nat.* **146,** 292–301.
2. Rundle, H. D., Nagel, L., Boughman, J. W. & Schluter, D. (2000) *Science* **287,** 306–308.
3. Johannesson, K. (2001) *Trends Ecol. Evol.* **16,** 148–153.
4. Schluter, D. (2000) *The Ecology of Adaptive Radiation* (Oxford Univ. Press, London).
5. Fryer, G. & Iles, T. D. (1972) *The Cichlid Fishes of the Great Lakes of Africa: Their Biology and Evolution* (Oliver and Boyd, London)
6. Kornfield, I. & Smith, P. F. (2000) *Annu. Rev. Ecol. Syst.* **31,** 163–196.
7. Turner, G. F., Seehausen, O., Knight, M. E., Allender, C. J. & Robinson, R. L. (2001) *Mol. Ecol.* **10,** 793–806.
8. Froese, R. & Pauly, D., eds. (2000) *Fishbase 2000* (International Center for Living Aquatic Resources Management, Makati City, Phillipines).
9. Kocher, T. D., Conroy, J. A., McKaye, K. R. & Stauffer, J. R. (1993) *Mol. Phys. Evol.* **2,** 158–165.
10. Albertson, R. C., Markert, J. A., Danley, P. D. & Kocher, T. D. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 5107–5110.
11. Konings, A. (2001) *Malawi Cichlids in Their Natural Habitat* (Cichlid Press, El Paso, TX), 3rd Ed.
12. Danley, P. D. & Kocher, T. D. (2001) *Mol. Ecol.* **10,** 1075–1086.
13. Seehausen, O., vanAlphen, J. J. M. & Witte, F. (1997) *Science* **277,** 1808–1811.
14. Knight, M. E., Turner, G. F., Rico, C., van Oppen, M. J. H. & Hewitt, G. M. (1998) *Mol. Ecol.* **7,** 1605–1610.
15. Van Doorn, G. S., Noest, A. J. & Hogeweg, P. (1998) *Proc. R. Soc. London Ser. B* **265,** 1915–1919.
16. Seehausen, O. & van Alphen, J. J. M. (1999) *Ecol. Lett.* **2,** 262–271.
17. Danley, P. D., Markert, J. M., Arnegard, M. E. & Kocher, T. D. (2000) *Evolution (Lawrence, Kans.)* **54,** 1725–1737.
18. Arnegard, M. E., Markert, J. A., Danley, P. D., Stauffer, J. R., Ambali, A. J. & Kocher, T. D. (1999) *Proc. R. Soc. London Ser. B* **266,** 119–130.
19. Markert, J. A., Arnegard, M. E., Danley, P. D. & Kocher, T. D. (1999) *Mol. Ecol.* **8,** 1013–1026.
20. Lande, R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3721–3725.
21. Dominey, W. (1984) in *Evolution of Fish Species Flocks*, eds. Echelle, A. A. & Kornfield, I. (Univ. of Maine Press, Orono), pp. 231–249.
22. Payne, R. J. H. & Krakauer, D. C. (1997) *Evolution (Lawrence, Kans.)* **51,** 1–9.
23. Turner, G. F. & Burrows, M. T. (1995) *Proc. R. Soc. London Ser. B* **260,** 287–292.
24. Masta, S. E. & Maddison, W. P. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 4442–4447.
25. Seehausen, O., van Alphen, J. J. M. & Witte, F. (1999) *Belg. J. Zool.* **129,** 279–294.
26. Holzberg, S. (1978) *Z. Zool. Syst. Evolut-forsch.*, **16,** 171–187.
27. Marsh, A. C., Ribbink, A. J. & Marsh, B. A. (1981) *Zool. J. Linn. Soc.* **71,** 253–264.
28. McKaye, K. R., Kocher, T., Reinthal, P., Harrison, R. & Kornfield, I. (1984) *Evolution (Lawrence, Kans.)* **38,** 215–219.
29. Van Oppen, M. J. H., Turner, G. F., Rico, C., Robinson, R. L., Deutsch, J. C., Genner, M. J. & Hewitt, G. M. (1998) *Mol. Ecol.* **7,** 991–1001.
30. Seehausen, O. & van Alphen, J. J. M. (1998) *Behav. Ecol. Sociobiol.* **42,** 1–8.
31. Higashi, M., Takimoto, G. & Yamamura, N. (1999) *Nature* **402,** 523–526.
32. Saetre, G. P., Moum, T., Bures, S., Kral, M., Adamjan, M. & Moreno, J. (1997) *Nature* **387,** 589–592.
33. Reinthal, P. N. & Meyer, A. (1997) in *Molecular Evolution and Adaptive Radiations*, eds. Givnish, T. J. & Sytsma, K. J. (Cambridge Univ. Press, Cambridge, U.K.), pp. 375–390.
34. Moran, P. & Kornfield, I. (1993) *Mol. Biol. Evol.* **10,** 1015–1029.
35. Rico, C., Bouteillon, P., Van Oppen, M. J. H., Knight, M. E., Hewitt, G. M. & Turner, G. F. (2003) *J. Evol. Biol.* **16,** 37–46.
36. Smith, P. F. & Kornfield, I. (2002) *Proc. R. Soc. London Ser. B* **269,** 2495–2502.
37. Ribbink, A. J., Marsh, B., Marsh, A., Ribbink, A. & Sharp, B. (1983) *S. Afr. J. Zool.* **18,** 149–310.
38. Spreinat, A. (1995) *Lake Malawi Cichlids From Tanzania* (Verduijn Cichlids, Zevenhuizen, The Netherlands).
39. Schraml, E. (1998) *African Cichlids I Malawi Mbuna* (A.C.S. Glaser, Mörfelden Walldorf, Germany).
40. Konings, A. (2001) *The Cichlids of Lake Malawi* (Cichlid Press, St. Leon-Rot, Germany).
41. Seehausen, O., van Alphen, J. J. M. & Lande, R. (1999) *Ecol. Lett.* **2,** 367–378.
42. Knight, M. E. (1999) Ph.D. thesis (Univ. of Southampton, Southampton, U.K.).
43. Vos, P., Hogers, R., Bleeker, M., Reijans, M., Vandelee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. & Zabeau, M. (1995) *Nucleic Acids Res.* **23,** 4407–4414.
44. Swofford, D. L. (2001) PAUP* Phylogenetic Analyses Using Parsimony (*and Other Methods) 4b8 (Sinauer, Sunderland, MA).
45. Hillis, D. M. & Huelsenbeck, J. P. (1992) *J. Hered.* **83,** 189–195.
46. Felsenstein, W. M. (2001) PHYLIP 3.6A2 ALPHA.
47. Fitch, W. M. & Margoliash, E. (1967) *Science* **155,** 279–284.
48. Maddison, W. P. & Maddison, D. R. (1992) MACCLADE: Analysis of Phylogeny and Character Evolution v3 (Sinauer, Sunderland, MA).
49. Mantel, N. (1967) *Cancer Res.* **27,** 209–220.
50. Briers, R. A. (1999) MANTEL XLA. VBA add-in for Microsoft EXCEL, Version 1.2.
51. Seehausen, O., Koetsier, E., Schneider, M. V., Chapman, L. J., Chapman, C. A., Knight, M. E., Turner, G. F., van Alphen, J. J. M. & Bills, R. (2003) *Proc. R. Soc. London Ser. B* **270,** 129–137.
52. Kornfield, I. (1991) *Genetics:* in *Cichlid Fishes: Behaviour, Ecology, and Evolution*, ed. Keenleyside, M. H. A. (Chapman & Hall, London), pp. 103–128.
53. van Oppen, M. J. H., Turner, G. F., Rico, C., Deutsch, J. C., Ibrahim, K. M., Robinson, R. L. & Hewitt, G. M. (1997) *Proc. R. Soc. London Ser. B* **264,** 1803–1812.
54. Carleton, K. L. & Kocher, T. D. (2001) *Mol. Biol. Evol.* **18,** 1540–1550.
55. Reimchen, T. E. (1989) *Evolution (Lawrence, Kans.)* **43,** 450–460.
56. Boughman, J. W. (2001) *Nature* **411,** 944–948.
57. Seehausen, O. (1996) *Lake Victoria Rock Cichlids, Taxonomy, Ecology, and Distribution* (Verduyn, Zevenhuizen, The Netherlands).
58. Ogden, R. & Thorpe, R. S. (2002) *Mol. Ecol.* **11,** 437–445.
59. Parson, Y. M. & Shaw, K. L. (2001) *Mol. Ecol.* **10,** 1765–1772.
60. Albertson, R. C., Streelman, J. T. & Kocher, T. D. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 5252–5257.