PROCEEDINGS OF THE ROYAL SOCIETY B

*Proc. R. Soc. B* (2006) **273**, 257–266
doi:10.1098/rspb.2005.3321
*Published online* 8 November 2005

# Colour-assortative mating among populations of *Tropheus moorii*, a cichlid fish from Lake Tanganyika, East Africa

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The species flocks of cichlid fishes in the East African Lakes Tanganyika, Malawi and Victoria are prime examples of adaptive radiation and explosive speciation. Several hundreds of endemic species have evolved in each of the lakes over the past several thousands to a few millions years. Sexual selection via colour-assortative mating has often been proposed as a probable causal factor for initiating and maintaining reproductive isolation. Here, we report the consequences of human-mediated admixis among differentially coloured populations of the endemic cichlid fish *Tropheus moorii* from several localities that have accidentally been put in sympatry in a small harbour bay in the very south of Lake Tanganyika. We analysed the phenotypes (coloration) and genotypes (mitochondrial control region and five microsatellite loci) of almost 500 individuals, sampled over 3 consecutive years. Maximum-likelihood-based parenthood analyses and Bayesian inference of population structure revealed that significantly more juveniles are the product of within-colour-morph matings than could be expected under the assumption of random mating. Our results clearly indicate a marked degree of assortative mating with respect to the different colour morphs. Therefore, we postulate that sexual selection based on social interactions and female mate choice has played an important role in the formation and maintenance of the different colour morphs in *Tropheus*, and is probably common in other maternally mouthbrooding cichlids as well.

**Keywords:** cichlid species flocks; *Tropheus moorii*; Lake Tanganyika; assortative mating; sexual selection

## 1. INTRODUCTION

The adaptive radiations of cichlid fishes in the East African Great Lakes Tanganyika, Victoria and Malawi remain the most astounding examples of morphologically, behaviourally and ecologically diverse species flocks (Fryer & Iles 1972; Stiassny & Meyer 1999). The intriguing diversity and species richness of the cichlid assemblages of these lakes, each of which comprises hundreds of endemic species that have evolved in the last several thousands to a few million years, established them as a prime model system for the study of evolution (Fryer & Iles 1972; Meyer *et al.* 1990; Stiassny & Meyer 1999; Kornfield & Smith 2000; Verheyen *et al.* 2003; Kocher 2004; Salzburger & Meyer 2004).

Adaptive radiation requires external physical events that provide the appropriate environment and, in many cases, intrinsic biological factors ('key innovations') that give particular lineages the potential to radiate (Simpson 1953; Schluter 2000). With regard to East African cichlids, the evolutionary success has been attributed to the interaction of different types of biological factors as well as the geological and climatological framework in which cichlid evolution has taken place (e.g. Sturmbauer 1998; Kornfield & Smith 2000). One hypothesis to explain the species richness of cichlids refers to the evolutionary significance of the adaptable pharyngeal jaw apparatus (Liem 1973). More recent studies have put forward the hypothesis that sexual selection through female choice of male (nuptial) coloration might have played an important role in the evolution of East African cichlids (Dominey 1984; Turner & Burrows 1995; Deutsch 1997; Seehausen & van Alphen 1999; Danley & Kocher 2001; Knight & Turner 2004). Speciation by disruptive sexual selection in cichlids has been proposed on the basis of field observations (van Oppen *et al.* 1998) as well as mate choice experiments in the laboratory (Knight *et al.* 1998; Seehausen *et al.* 1999; Knight & Turner 2004). Further evidence for sexual selection as an important isolating mechanism comes from the observable breakdown of visual reproductive barriers under monochromatic light conditions or under turbid waters resulting from human inference (Seehausen *et al.* 1997). The occurrence of assortative mating in natural cichlid populations has so far not been surveyed at a larger scale using population genetic methods.

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The electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2005.3321 or via http://www.journals.royalsoc.ac.uk.

*Received* 16 August 2005
*Accepted* 5 September 2005

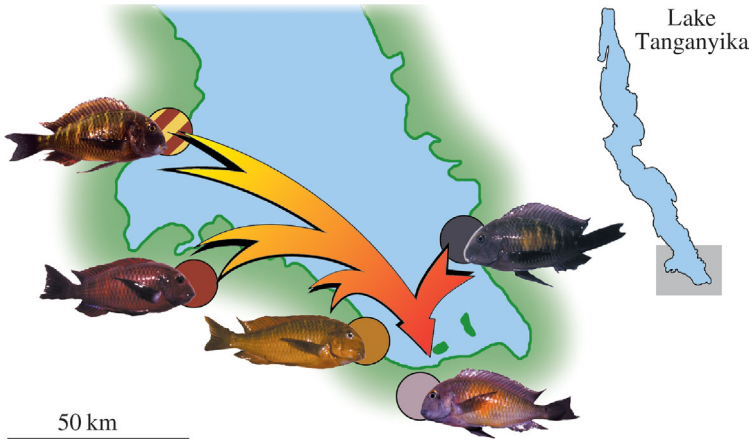


Figure 1. Human-induced secondary admixis due to faunal translocation of several non-indigenous southern populations into a harbour bay in Mpulungu, Zambia. The pictures of the fishes were taken in the field. The fishes were placed in their hypothesized source localities.

Here, we report on a human-mediated admixis of differentially coloured *Tropheus moorii* populations that were put in sympatry in a small bay in the very south of Lake Tanganyika. The genus *Tropheus* is in need of taxonomic revision (Snoeks *et al.* 1994). More than 100 differentially coloured morphs can be distinguished in *T. moorii*, with almost every continuous stretch of rocky shoreline of Lake Tanganyika being inhabited by its own local colour morph (Sturmbauer & Meyer 1992; Baric *et al.* 2003; Schupke 2003; Sturmbauer *et al.* 2005). Both males and females typically display the same coloration, while juveniles show a more cryptic, often striped, phenotype. Despite marked differences in coloration and, often, considerable genetic distances between populations (Sturmbauer & Meyer 1992; Baric *et al.* 2003; Sturmbauer *et al.* 2005), *T. moorii* occupy the same ecological niche all around Lake Tanganyika. Also, their overall morphology (except coloration) is highly similar, and no differences in breeding behaviour and spawning time have been observed in *T. moorii* (Sturmbauer & Meyer 1992; Baric *et al.* 2003; Schupke 2003). Therefore, it has been argued that in *T. moorii* ecology and morphology are under stabilizing selection, while coloration appears to be a sexually selected trait driving allopatric speciation under morphological stasis (Sturmbauer & Meyer 1992).

In June 1998, local fishermen collected about 300 adult individuals of *T. moorii* from several sites in the southern part of the lake, with the intention of exporting these colourful fishes for the aquarium trade. Owing to the fact that the fishing was carried out without concession, Zambian authorities refused export permits. Instead of returning the fishes to their original habitats, as instructed by the local authorities, the catch was released in a small harbour basin of not more than 200 $m^2$ in size in front of the Fisheries Department in Mpulungu, Zambia (L. Mwape 1999, personal communication).

This artificial amalgamation of several differently coloured populations of *T. moorii* (figure 1) provided the unique opportunity to study the biological interactions between several colour morphs of the same cichlid species in their natural environment. It also resembles a situation as it may have existed during previously reported major low water levels of Lake Tanganyika, which led to the admixis of several formerly isolated populations (Sturmbauer 1998; Kornfield & Smith 2000; Sturmbauer *et al.* 2001). We analysed DNA sequences of the first section of the mitochondrial control region (442 bp) as well as five microsatellite loci from 457 specimens sampled consecutively over a period of three years from the Mpulungu harbour bay. We tested whether or not members of the same colour morph mate assortatively and to what extent hybridization had occurred between different colour morphs in the admixed population, applying both maximum-likelihood-based parenthood analyses (Marshall *et al.* 1998; Slate *et al.* 2000) and Bayesian inference of population structure (Pritchard *et al.* 2000).

Table 1. Number of specimens of *Tropheus moorii* collected for this study in the three years of sampling (1999, 2000 and 2001). (Based on the size of each individual and growth rates for this species (Egger *et al.* 2004), we distinguished between adults that were present in the harbour bay before, or were introduced in the course of, the admixis, and offspring that were sired after admixis. The reference population was collected about 50 m away from the harbour bay in Mpulungu.)

| | 1999 | 2000 | 2001 | total |
|---|---|---|---|---|
| adults | 73 | 58 | 19 | 150 |
| offspring | 22 | 102 | 88 | 212 |
| reference population | 23 | 29 | 43 | 95 |
| total | 118 | 189 | 150 | 457 |

## 2. MATERIAL AND METHODS

### (a) *Sampling*

Sampling was carried out in February 1999, 2000 and 2001, which corresponds to 0.5, 1.5 and 2.5 years after the release of non-indigenous specimens in the harbour bay of Mpulungu, Zambia. Fishes were collected with gill nets, weighed, sized, sexed and photographed (table 1). Finally, a piece of the caudal fin was taken for DNA extraction and preserved in 100% ethanol before the individuals were released. An adjacent population, situated about 50 m away from the admixed population, was sampled as reference group, leading to a total of 457 specimens (see table S1, in the electronic supplementary material).

### (b) *Morphological data*

A principal component analysis (PCA) was carried out among individuals whose total length (TL) was more than

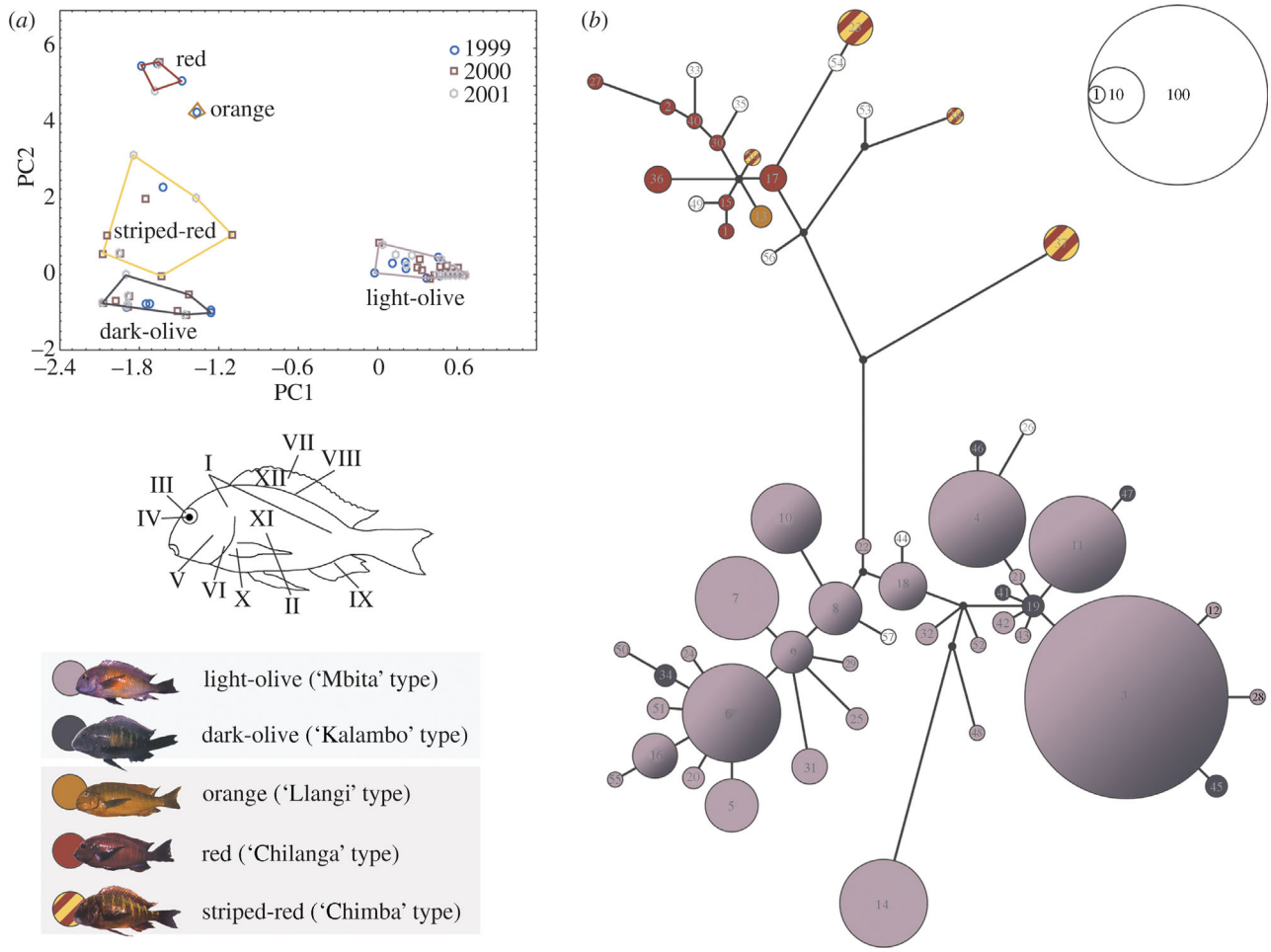


Figure 2. Morphological and genetic classification of the studied specimens of *Tropheus moorii*. (*a*) The principal component analysis based on 12 landmarks related to coloration (I–XII) identified five classes of individuals: 'light-olive', 'dark-olive', 'orange', 'red' and 'striped-red'. The fishes of the reference population were all classified as 'light-olive'. (*b*) Unrooted haplotype network including all 56 mitochondrial haplotypes found. The length of the branches interrelating the haplotypes reflects the genetic distance as the number of mutations. The sizes of the circles correspond to the number of individuals that share a particular haplotype; the number in each circle refers to table S1 that is in the electronic supplementary material. The colours of the circles refer to the morphological classification of the individual(s) of that particular haplotype. Shared haplotypes between colour morphs were only found between 'light-olive' and 'dark-olive' phenotypes. Open circles represent juveniles for which no colour information was available.

60 mm ($n=360$), since juveniles of *T. moorii* of less than about 60 mm in length display an almost indistinguishable stripe pattern. For the reference population, only adult individuals of the first year of sampling were included in this analysis. Twelve features related to coloration were used for this analysis (see figure 2*a*): overall body colour (red/light(=light olive)/dark(=dark olive)/orange), central body colour (red/yellow/dark/orange), colour of eyelid (red/light/dark/yellow), eye-ring (blue/dark/light), operculum (red/dark/light/blue), operculum edge (red/light/dark/yellow/orange/blue), dorsal fin (dark-red/light/dark/light-blue/light-red), base of dorsal fin (dark-red/light/dark/orange/blue/light-red), base of anal fin (dark-red/light/dark/orange/blue/light-red), base of pectoral fin (dark-red/light/dark/orange/blue/light-red), stripe/dot pattern (uniform/stripe/dot) and dorsal fin pattern (uniform/striped). Characters were translated into a binary data matrix and analysed in a PCA.

### (c) *Molecular methods*

Total DNA was extracted from fin-clips preserved in 100% ethanol applying a proteinase K digestion followed by sodium chloride extraction and ethanol precipitation (Bruford *et al.* 1998). PCR amplification was performed according to standard methods on an ABI 9700 thermocycler (Applied Biosystems) for the mitochondrial DNA, and on an air thermocycler (Idaho Technologies) for the microsatellite loci. We used published primers for both the amplification of a 442 bp segment of the mitochondrial control region (Kocher *et al.* 1989; Salzburger *et al.* 2002), as well as for the five microsatellite loci Pzeb3, TmoM11, TmoM25, ULI2 and UME002 (forward primers were labelled with fluorescent dyes FAM or HEX; Parker & Kornfield 1996; Zardoya *et al.* 1996; van Oppen *et al.* 1997). The PCR fragments of the control region were purified using the ExoSAPIT enzyme mix (USB), directly sequenced with the BigDye sequencing chemistry (Applied Biosystems), and analysed on an ABI3700 automatic sequencer (Applied Biosystems). The microsatellite loci were analysed on the same device using the internal size marker Genescan 500 (Applied Biosystem).

### (d) *Data analyses*

DNA sequences were aligned using SEQUENCHER 4.0 (Genecodes) and genetically characterized based on a large body of published data from *T. moorii* (Sturmbauer & Meyer

1992; Baric *et al.* 2003; Sturmbauer *et al.* 2005). A haplotype network was constructed with the computer program TCS (Clement *et al.* 2000). In the case of alternative branching orders, those ones were chosen that were indicated by a maximum-likelihood analysis in PAUP*4.0b10 (Swofford 2002), after translating the maximum-likelihood tree into an unrooted network. We then mapped the colour information of all mitochondrial haplotypes, for which colour data were available, onto the haplotype network.

For the microsatellite data, we estimated allele frequencies for every locus using Arlequin 2.1 (Schneider *et al.* 1999). Departure from Hardy–Weinberg expectations for every locus was tested within the different partitions of our dataset using a test analogous to Fisher's exact test (Guo & Thompson 1992), as implemented in Arlequin. Linkage disequilibrium for all pairs of loci was tested for the same partitions with Arlequin. Genetic differentiation between the four groups, 'light-olive', 'dark-olive', 'red' and 'striped-red', was measured with Wright's *F*-statistics ($F_{ST}$; Weir & Cockerham 1984) as implemented in Arlequin (because of its rare occurrence, 'orange' was not included). This analysis was performed with both microsatellite and mitochondrial control region data. Critical significance levels for multiple testing were corrected following the sequential Bonferroni procedure (Rice 1989).

Parenthood tests were carried out for all specimens that were born after admixis of the differentially coloured populations. The classification of these individuals was based on growth rates for exactly this population (Egger *et al.* 2004). These growth rates of *T. moorii* were delineated from a mark–recapture study in the Mpulungu harbour using chemical tagging of otoliths to examine increment formation. Accordingly, for the 1999 sample, individuals of less than 65 mm TL were considered as juveniles conceived after secondary admixis; for the 2000 sample, individuals of less than 78 mm TL were included; and for the 2001 sample, individuals of less than 82 mm TL were defined as juveniles born in the admixed population. Maximum-likelihood-based parenthood tests were initially performed using the computer program Cervus 2.0 (Marshall *et al.* 1998; Slate *et al.* 2000), with all individuals being considered as potential parents. The probabilistic parent–offspring relationships proposed by Cervus were further evaluated by computing likelihood ratios for short tandem repeats (STRs) (see Hammnond *et al.* 1994). The additional information provided by the mitochondrial DNA sequences was accounted for using Bayes' theorem. To distinguish between real candidate parents and siblings that also could be identified as most likely parent(s), we used the information on sampling date and total length of each individual to discern parentage.

As a complementary approach to the morphological evaluation of recognized parent–offspring pairs, which excludes all individuals that either lack colour information or that have unknown parents, we performed a population assignment test. To this end, we used a Bayesian clustering method implemented in Structure 2.1 (Pritchard *et al.* 2000) using the microsatellite data and coding the mitochondrial haplotypes as a sixth marker. Here, our strategy was not to infer the population structure and the number of distinct clusters (*K*) from the genetic data available (default mode in Structure). Instead, we used the information provided by the morphological evaluation of the adult specimens based on their coloration as a prior in the 'learning phase' (burn in), in order to test the genetic assignment of the offspring individuals. This has been shown to be an accurate tool to detect immigrants (e.g. Pritchard *et al.* 2000) and/or to identify hybrid individuals (e.g. Beaumont *et al.* 2001). We ran Markov chain Monte Carlo simulations with 500 000 replications (burn in = 50 000; admixture model with prior population information; correlated allele frequencies; PCA results as prior population information) for *K* (number of genetic clusters) = 5 (based on the five phenotype groups 'light-olive', 'dark-olive', 'orange', 'red' and 'striped-red').

We then used the results from the parenthood tests and from the genetic assignment to calculate the ratio of intra-morph mating events versus inter-morph matings, and compared this ratio to the expected ratio under the assumption of random mating using a chi-square goodness-of-fit test.

## 3. RESULTS

PCA demonstrated the existence of five groups of distinctly coloured individuals in the admixed population ('light-olive', 'dark-olive', 'orange', 'red' and 'striped-red' in figure 2*a*). Typically, for the analysis of presence/absence data, a large number of principal components are needed to account for most of the variation (Manly 1994). In this case, however, the main groupings showing the variability we were interested in were evident in the first two axes. All adult fishes from the reference population sampled in the first year after admixis were grouped within the 'light-olive' phenotype confirming this phenotype as the indigenous one. These fishes resemble the 'Mbita' type, named after the locality from where these fishes were initially exported for the aquarium trade. Although data on the exact sampling localities for the introduced specimens were not available, most of the fishes could be assigned to populations from a specific stretch of shoreline based on their characteristic colorational morphotype and their mitochondrial haplotype (Sturmbauer & Meyer 1992; Baric *et al.* 2003; Schupke 2003; Sturmbauer *et al.* 2005; see figure 1). Based on their coloration, the dark-olive individuals are likely to have originated from populations on the Zambian east coast of Lake Tanganyika (morphologically resembling individuals that are found between Kala and Chituta Bay). Likewise, the orange ('Ilangi' type), red ('Chilanga' type) and striped-red ('Chimba' type) individuals were assigned to the shoreline northwest of the Lufubu estuary, between Cape Inangu and Moliro.

A total of 56 mitochondrial haplotypes was observed, of which 19 were clearly distinct and occurred exclusively in the orange, red and striped-red individuals (figure 2*b*). Seven out of the 21 haplotypes of the reference group were not present in the admixed population. Mitochondrially, all light- and dark-olive individuals including the reference population were grouped with DNA sequences previously assigned to lineage A2 according to Baric *et al.* (2003; equivalent to TCS-1 in Sturmbauer *et al.* 2005). The individuals with orange or red phenotypes, and all but one striped-red fish clustered within lineage F (Baric *et al.* 2003; TCS-7 in Sturmbauer *et al.* 2005). This single striped-red individual, resolved within lineage G (Baric *et al.* 2003; TCS-8 in Sturmbauer *et al.* 2005), most likely stems from the population at Katoto, which was previously characterized as heterogeneous due to admixis, comprising intermediate phenotypic traits and three

Table 2. Pairwise $F_{ST}$-values between four different colour morphs of *Tropheus moorii* that were obtained by the principal component analysis based on coloration landmarks (see figure 2*a*). (The $F_{ST}$-values above the diagonal are based on mitochondrial control region sequences, and those below the diagonal are based on the microsatellite data. For the reference population, only specimens from the first year of sampling were included, which was due to the existence of immigrants and potential hybrids in the later samples. All values are corrected by means of the sequential Bonferroni technique. n.s., non-significant, $p \geq 0.05$; *significant, $p < 0.05$; **significant, $p < 0.001$.)

| | dark-olive | light-olive | red | striped-red | reference |
|---|---|---|---|---|---|
| dark-olive | — | $0.0066^{n.s.}$ | $0.7754^{**}$ | $0.7531^{**}$ | $0.0000^{n.s.}$ |
| light-olive | $0.0013^{n.s.}$ | — | $0.7443^{**}$ | $0.7291^{**}$ | $0.009\,43^{n.s.}$ |
| red | $0.1342^{**}$ | $0.1227^{**}$ | — | $0.0000^{n.s.}$ | $0.7580^{**}$ |
| striped-red | $0.0704^{**}$ | $0.0641^{**}$ | $0.0073^{n.s.}$ | — | $0.7439^{**}$ |
| reference | $0.0140^{*}$ | $0.0092^{n.s.}$ | $0.1056^{**}$ | $0.0487^{**}$ | — |

mitochondrial haplotype lineages (A2, A4 and G; Baric *et al.* 2003; Sturmbauer *et al.* 2005).

*F*-statistics based on the mitochondrial DNA sequences and microsatellite data revealed no significant population differentiation between the groups of light- and dark-olive phenotype (table 2), which might be due to the origin of several individuals from populations such as Katoto that contain individuals assigned to both colour groups. Although distinct in coloration, the groups of red and striped-red individuals were not genetically differentiated from each other. All remaining pairwise $F_{ST}$ comparisons revealed significant population differentiation. The individuals of the adjacent reference population showed, based on the microsatellites, no significant population differentiation from the light-olive phenotypes (the indigenous group of the harbour bay), while they were distinct from all other groups (table 2).

Based on the microsatellite data, no linkage disequilibrium was found in the dark-olive fish and the reference population. In the light-olive individuals, 4 out of 20 pairwise comparisons indicated linkage disequilibrium, which is most likely due to the fact that at least one introduced population plus the indigenous fishes were grouped into this cluster. Two loci each were found to be linked to the red (ULI2 and UME002) and striped-red (TmoM11 and UME002) individuals. However, both groups contained a relatively small number of individuals. Also, it is not known whether all red and striped-red fish originated from only one original population each. In the juvenile individuals sired after admixis, 14 out of 20 pairwise comparisons were indicative of a linkage disequilibrium. Similarly, three loci showed a significant heterozygote deficit in the juvenile individuals, while in the dark-olive, red and striped-red fish no deviation from Hardy–Weinberg equilibrium was found. In the light-red and the reference population, a heterozygote deficit was found in one locus (TmoM25).

The parenthood tests targeting the individuals sired after the secondary admixis using all specimens of the admixed population as candidate parents identified parents for 52 individuals. For 41 individuals one, and for 11 individuals both, parents were determined. Forty-one (78.9%) of these offspring individuals displayed the same phenotype as their parents. However, three light-olive individuals were assigned to a dark-olive parent (5.7%), and seven dark-olive individuals (13.5%) were likely to have a light-olive parent indicating several mating events between these two groups. One red offspring individual (1.9%) was likely to have had an orange mother.

The microsatellite-based Bayesian inference of population structure (Pritchard *et al.* 2000) provided a more detailed picture of the genetic composition of the admixed population's individuals (figure 3). The light-olive phenotypes of the admixed assemblage were grouped into one genetic cluster (class A genotypes; figure 3*a*). Likewise, the dark-olive individuals were clustered together (class B genotypes; figure 3*b*). All red, orange and striped-red phenotypes were genetically resolved into a single group (class C genotypes; figure 3*c–e*). A fourth class of genotypes was found primarily in the reference population (class D genotypes; figure 3*f*), a fifth group was determined based on genetic grounds but was not recognized morphologically. These class E genotypes (yellow bars in figure 3) could possibly represent a cryptic population among light-olive phenotypes from an originally isolated spot that was released with all the other non-indigenous individuals. As additional information about class E genotypes was not available, these were excluded from further analyses.

About 63% (114 out of 180) of the offspring in the admixed population (figure 3*g*) were assigned to a particular genotype class with a probability $P_a$ of more than 0.75, which we considered as juveniles of intra-genotypic matings. The value for $P_a > 0.75$ seems rather high, given the expected probability of about 0.5 for an (F1-) hybrid individual. However, we chose the value $P_a > 0.75$ for intra-genotypic matings based on the observation that, in our case, with this threshold an alternative (hybrid) origin of a tested juvenile could be ruled out in more than 90% of the cases (based on the upper bound of the probability interval). In addition to these 63% of the offspring, 5% of the offspring individuals (9 out of 180) were assigned to matings between class A and class D genotypes (reference group), which show the same coloration (light-olive), and, thus, were also considered as offspring from intra-morph matings. Accordingly, 68.3% (123 out of 180) of offspring originated from within morph matings. This is significantly more than could be expected under the assumption of random mating ($\chi^2_1 > 6.64$; $p < 0.01$). Only three individuals (1.6%) originated from matings between 'olive' and 'reddish' parents, which is equivalent with matings between different mitochondrial haplotype groups. This number is significantly smaller than could be expected under random conditions ($\chi^2_1 > 6.64$; $p < 0.01$).

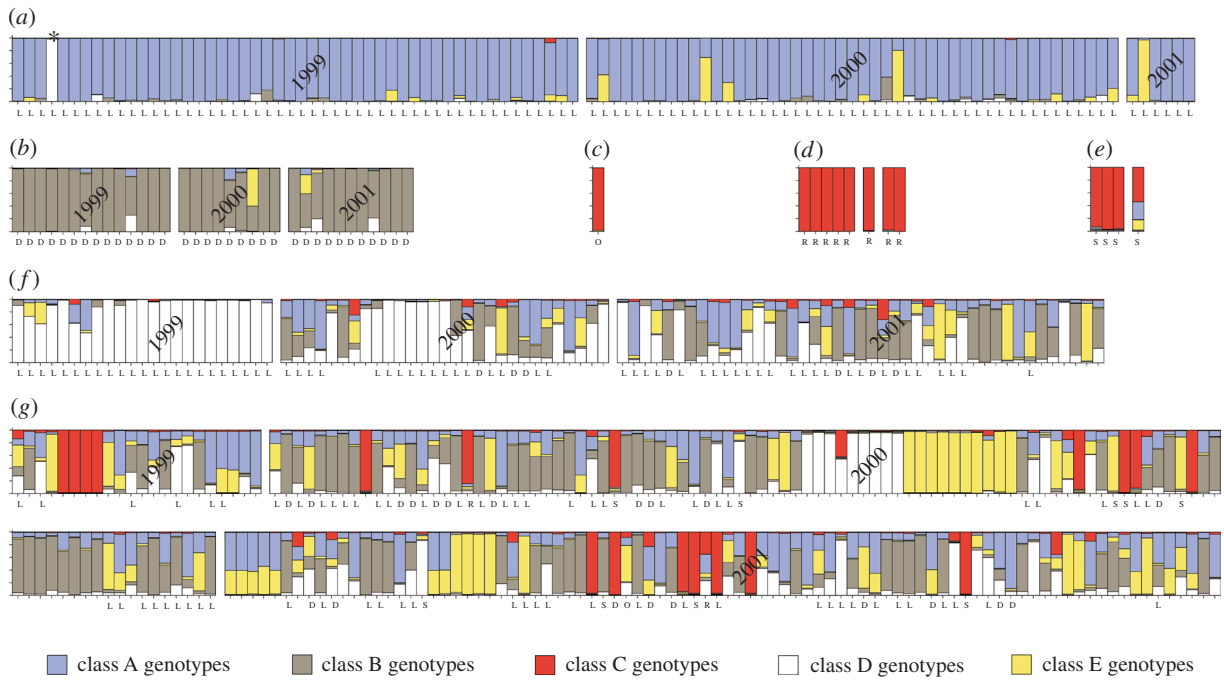


Figure 3. Results of the population assignment test using STRUCTURE 2.1 (Pritchard *et al.* 2000) based on the information provided by five microsatellite markers and the mitochondrial haplotype. Each bar represents a single individual, the probability ($P_a$) for its assignment to one of the five classes of genotypes is shown as the proportion of the bar illustrated by the respective colour. As far as a specific phenotype could be assigned to an individual (see figure 2), this information has been depicted as 'L' for light-olive, 'D' for dark-olive, 'O' for orange, 'R' for red and 'S' for striped-red. (*a*) The light-olive phenotypes of the admixed population were almost exclusively assigned to one genotype class (class A). (*b*) Similarly, the dark-olive phenotypes were assigned to a second genotype class (B). (*c*) The orange, (*d*) the red and (*e*) the striped-red individuals were all clustered into the same group of genotypes (class C). (*f*) Within the reference population, homogenous clustering could only be detected in the first year of sampling (class D genotypes). In the second and, particularly, in the third year of sampling, the reference population consisted largely of migrants and hybrid individuals. (*g*) Individual population assignment for all juveniles sired after the introduction of non-indigenous fishes. A fifth group of individuals (class E genotypes) was found, which might be a cryptic population not detected by other methods. The asterisk marks one specimen of light-olive phenotype sampled in 1999 that most likely represents a migrant from the reference population.

## 4. DISCUSSION

Cichlid fishes are prime examples where 'selective mating' (Kosswig 1947) is believed to have played an important role in speciation processes. In the Central American midas cichlid (*Amphilophus citrinellus*), for example, assortative mating has been demonstrated by means of laboratory experiments and field observations (McKaye & Barlow 1976; Barlow 1977; Barlow & Rogers 1978), and it has been argued that this form of sexual selection is the main force maintaining the colour polymorphism of a cryptic, striped 'normal' and a conspicuous 'golden' morph (McKaye 1980; Barlow 1983, 2000). Similarly, disruptive sexual selection via colour-assortative mating has been suggested as an important factor that is at least partially responsible for the evolution of the myriad of cichlid species in the East African Great Lakes (e.g. Dominey 1984; McKaye 1991; Danley & Kocher 2001; Allender *et al.* 2003). More than 100 different colour morphs of *T. moorii* are among the most famous examples, where sexual selection on colour traits is likely to have triggered diversification (Sturmbauer & Meyer 1992; Sturmbauer 1998; Schupke 2003). Still, *T. moorii* is somewhat outstanding among the haplochromine cichlids that include the Tropheini (e.g. Salzburger *et al.* 2002, 2005), in that it does not show sex-based colour polymorphism. *Tropheus moorii* displays a complex mating system, in which colour is not only likely to be involved in sexual selection, but also in the context of intraspecific social interactions—regardless of sex (Sturmbauer & Meyer 1992; Sturmbauer & Dallinger 1995; Sturmbauer 1998; Schupke 2003). Thus, assortative mating based on coloration is likely to act similarly to that found in the haplochromine cichlids from Lakes Victoria and Malawi providing the basis of population differentiation and speciation (e.g. Dominey 1984; Turner & Burrows 1995; Deutsch 1997; Seehausen & van Alphen 1999; Danley & Kocher 2001; Knight & Turner 2004; Kocher 2004), with the additional feature of being critical for social interactions within populations. The lack of sexual colour dichromatism in *Tropheus* is explained by the importance of sex-independent social signals.

The parenthood analyses for the offspring of the admixed *T. moorii* populations revealed that about 80% of the positively typed individuals had the same phenotype as their probable parent(s), suggesting a marked degree of assortative mating with respect to the different colour morphs. This ratio of within-population fertilization is higher than the one observed in laboratory trials with five populations of *Pseudotropheus zebra* from Lake Malawi (Knight & Turner 2004). However, in more than half of the cases our sampling in the Mpulungu harbour bay recovered only one parent, so that the possibility remains that the second (unknown) parent might belong to a different colour morph.

Our second approach, the population assignment test, overcomes the problem of unknown parent or offspring

phenotypes, as it clusters individuals according to their genotypes only. Given that the recovered genotype classes are in agreement with phenotypic clusters, offspring of within-colour-morph matings should have a high probability of being assigned to only one genotype class (the one to which both parents belong), while the genome of hybrids between colour morphs should carry portions of both parental genotype classes. In our case, the population assignment tests (Pritchard *et al.* 2000) recovered the adult individuals with light- and dark-olive phenotypes as well as the reference group (light-olive phenotype) as distinct genotype classes. The red, orange and striped-red individuals were, however, grouped into a single genotype class (figure 3). Based on these assignments, about 68% of the offspring of the admixed individuals are likely to be derived from within-colour-morph matings ($P_a > 0.75$), which is significantly more than could be expected under random mating ($p < 0.01$). This value is similar to the frequency of within-population matings found in laboratory trials with *P. zebra* from Lake Malawi (Knight & Turner 2004).

Most inter-genotypic matings occurred between individuals assigned to class A and class B genotypes, which correspond to light- and dark-olive phenotypes, respectively. This is also reflected in the low $F_{ST}$-value when comparing these two clusters. In their experiments with *P. zebra*, Knight & Turner (2004) observed that two populations, which were not the most closely related ones, did not mate assortatively, and they interpreted this as due to the relative morphological resemblance of these populations with respect to each other. In our natural set-up, the light- and dark-olive phenotypes differ primarily in the main body coloration and both groups show a more or less pronounced yellow blotch on their flanks (figures 1 and 2). They also fall into the same mitochondrial haplotype cluster (figure 2), which implies that they are, in fact, more closely related compared to the reddish ones. The parenthood analyses also recovered one mating event between red and orange fish. This is not surprising based on their genetic similarity and the morphological resemblance with red coloration. Since their genotypes were clustered—together with the striped-red fish—into one genetic cluster (class C genotypes), no further evaluation of matings between these three phenotype groups was possible. The most striking result was that only three out of all 180 juveniles were found to be 'hybrids' between 'olive' and 'reddish' parents and, hence, between two different mitochondrial haplotype lineages, pointing to a marked degree of assortative mating. The occurrence of assortative mating is further supported by the observed deviation from Hardy–Weinberg equilibrium as well as the linkage disequilibrium in the juvenile individuals that were sired after admixis.

In spite of the philopatric behaviour of *Tropheus*, the genetic consequences of the human-induced secondary admixis were not restricted to the harbour bay, where the fishes had been released. The reference population, which is situated about 50 m away from the location of admixis, turned out to be greatly affected as well. While in the first year of sampling the specimens of the reference population consisted exclusively of class D genotypes (white bars in figure 3*f*), the frequency of these indigenous genotypes decreased over the sampling period so that in the third year of sampling more than 80% of the reference population consisted of introgressed genotypes or hybrids between introgressed and indigenous fishes (figure 3*f*). A morphological evaluation of these introgressants or hybrids was not possible, since a large number of the non-indigenous genotypes belong to class A corresponding to the light-olive phenotypes, which are morphologically indistinguishable from the reference population's fish. Also, many individuals collected there in 2000 and 2001 were smaller than 60 mm and, therefore, not suitable for morphological analyses.

That migration between adjacent populations occurs naturally can be detected based on the low $F_{ST}$-values between the reference population's individuals and the light-olive individuals (table 1) as well as in one individual in the light-olive phenotypes of the admixed population that genetically belongs to the reference group (marked by an asterisk in figure 3*a*), and thus most likely represents an immigrant from the reference group before admixis. However, natural migration seems to have occurred at rather low frequencies compared to the rapid dispersal of introduced genotypes into the reference population after admixis. The high density of individuals that must have persisted in the small harbour bay after the introduction of 300 individuals might be the driving force for migration of introduced individuals into adjacent rocky habitats.

Our approach combining parenthood analyses, population pairwise $F_{ST}$-values and population assignment tests revealed that complex interactions take place between allopatric colour morphs of *T. moorii* when put in sympatry in their natural environment. For the first time, our study shows that colour morphs of *T. moorii*, which do not have any contact in nature, mate assortatively in the presence of other colour morphs. Assortative mating appears to be even stronger between the two morphologically and genetically redefined clusters 'olive' and 'reddish'. Assortative mating, i.e. the observation that individuals of two or more classes tend to breed with their own kind, may be based on different features in fishes. In the case of the generally colourful cichlid fishes (coloration is why they are called *Buntbarsche* in German), body coloration is generally accepted to be the decisive feature of mate choice (McKaye 1980, 1991; Barlow 1983, 2000; Dominey 1984; Turner & Burrows 1995; Danley & Kocher 2001; Allender *et al.* 2003)—at least in maternal mouthbrooders. Other examples for assortative mating in fishes involve exaggerated male traits such as the elongated caudal fins of male swordtails of the genus *Xiphophorus* that are selected for through female choice (Ryan & Wagner 1987; Basolo 1990, 1995), chemical (olfactory) cues (e.g. McLennan & Ryan 1999), differences in spawning time and/or locality (e.g. Hendry *et al.* 2004; Østbye *et al.* 2004; Fraser & Bernatchez 2005; Hendry & Day 2005; McLean *et al.* 2005), or size (e.g. McLean *et al.* 2005). Given that there are no detectable differences in overall morphology, ecology, spawning behaviour and timing in *T. moorii*, and since local differences in natural selection are improbable, we consider colour-assortative mating based on female preferences as the most likely explanation for our finding of a marked degree of colour-assortative mating in *T. moorii*. Also, it seems unlikely that post-zygotic mechanisms (such as reinforcement) account for our observation, since all the many crossings between different colour morphs of *Tropheus* reported so far produced fully viable hybrid offspring, and clutch sizes were not smaller

(e.g. Schupke 2003). Still, the possibility remains that chemical or acoustic cues, for which we did not test, play a role in social interactions in *T. moorii*. Future studies should also address the question of whether or not genetic drift was associated with the evolution of different colour patterns and divergent female preferences in cichlid populations by breaking up the linkage equilibrium between loci involved in colour patterning and female preference.

Taken together, our results support the hypothesis that colour-assortative mating played an important role in the formation and, later, in the maintenance of the different colour morphs as well as the major genetic lineages in *Tropheus* (Sturmbauer & Meyer 1992). The data also imply that the degree of reproductive isolation could be a function of time since divergence, since almost all (more than 95%) mating events involving two different colour morphs happened within and not between the mitochondrial haplotype groups (as defined in Baric *et al.* 2003; Sturmbauer *et al.* 2005). This indicates that assortative mating might have evolved gradually in the allopatric populations of *T. moorii*. Thus, (colour-) assortative mating may not only be a causal factor driving sympatric speciation—as proposed for the cichlid species flocks in Lakes Victoria and Malawi (e.g. Turner & Burrows 1995; Kornfield & Smith 2000; Danley & Kocher 2001)—but may as well happen after secondary admixis, whenever colour differences evolved in allopatry before. In this way, sexual selection drives colour divergence in allopatry, to lead gradually to speciation whenever the differences become large enough to influence mate choice (Greenwood 1965; Coyne & Orr 2004; Knight & Turner 2004). It is interesting to note that several authors have argued that sympatric speciation by sexual selection alone is rather unlikely (e.g. Arnegard & Kondrashov 2004; Coyne & Orr 2004). Further experiments, including laboratory mating trials, are currently underway to detail our understanding of assortative mating in the cichlid genus *Tropheus*.

We would like to dedicate this work to the late Leonard Mwape. We are grateful to our helpers in the field S. Baric, B. Egger, R. Hanel, B. Kirchberger, S. Koblmüller, L. Makasa, G. Musuluta, M. Nabuyanda, C. Rhomberg, R. Shapola; the divers T. Chempatika, A. and L. Musonda, E. Shichizima, W. Simanwe, P. Simuteonde, J. Simuyemba, K. Sudi; and the staff at the Mpulungu Station, Department of Fisheries, Ministry of Agriculture and Cooperatives, Republic of Zambia. We are further grateful to P. Bunje, M. Barluenga, A. Meyer, K. Sefc, two anonymous reviewers and the Editorial Board Member for valuable comments on the manuscript. This research was conducted in the framework of the Agreement of Understanding between the University of Zambia at Lusaka, the Department of Fisheries, Ministry of Agriculture and Cooperatives, Zambia, and the University of Innsbruck (Austria), the University of Berne (Switzerland) and the University of Graz (Austria). Funding was received from the Austrian Science Foundation (project 12339 and 15239-GEN). W.S. was supported by a DOC fellowship of the Austrian Academy of Sciences and a Marie-Curie fellowship of the EU.

## REFERENCES

Allender, C. J., Seehausen, O., Knight, M. E., Turner, G. F. & Maclean, N. 2003 Divergent selection during speciation of Lake Malawi cichlid fishes inferred from parallel radiations in nuptial coloration. *Proc. Natl Acad. Sci. USA* **100**, 14 074–14 079. (doi:10.1073/pnas.2332665100)

Arnegard, M. E. & Kondrashov, A. S. 2004 Sympatric speciation by sexual selection alone is unlikely. *Evolution* **58**, 222–237.

Baric, S., Salzburger, W. & Sturmbauer, C. 2003 Phylogeography and evolution of the Tanganyikan cichlid genus *Tropheus* based upon mitochondrial DNA sequences. *J. Mol. Evol.* **56**, 54–68. (doi:10.1007/s00239-002-2380-7)

Barlow, G. W. 1977 Incompatibility and assortative mating in the midas cichlid. *Behav. Ecol. Sociobiol.* **2**, 49–59. (doi:10.1007/BF00299288)

Barlow, G. W. 1983 The benefits of being gold: behavioral consequences of polychromatism in the midas cichlid, *Cichlasoma citrinellum*. *Environ. Biol. Fishes* **8**, 235–247. (doi:10.1007/BF00001089)

Barlow, G. W. 2000 *The cichlid fishes. Nature's grand experiment in evolution*. Cambridge, MA: Perseus Publishing.

Barlow, G. W. & Rogers, W. 1978 Female midas cichlids' choice of mate in relation to parents' and to own colour. *Biol. Behav.* **3**, 137–145.

Basolo, A. L. 1990 Female preference predates the evolution of the sword in swordtail fish. *Science* **250**, 808–810.

Basolo, A. L. 1995 Phylogenetic evidence for the role of a preexisting bias in sexual selection. *Proc. R. Soc. B* **259**, 307–311.

Beaumont, M., Barratt, E. M., Gottelli, D., Kitchener, A. C., Daniels, M. H., Pritchard, J. K. & Bruford, M. W. 2001 Genetic diversity and introgression in the Scottish wildcat. *Mol. Ecol.* **10**, 319–336. (doi:10.1046/j.1365-294x.2001.01196.x)

Bruford, M. W., Hanotte, O., Brookfield, J. F. Y. & Burke, T. 1998 Multi-locus and single-locus DNA fingerprinting. In *Molecular analysis of populations* (ed. A. R. Hoelzel), pp. 283–336. New York: Oxford University Press.

Clement, M., Posada, D. & Crandall, K. A. 2000 TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* **9**, 1657–1659. (doi:10.1046/j.1365-294x.2000.01020.x)

Coyne, J. A. & Orr, H. A. 2004 *Speciation*. Sunderland, MA: Sinauer Associates.

Danley, P. D. & Kocher, T. D. 2001 Speciation in rapidly diverging systems: lessons from Lake Malawi. *Mol. Ecol.* **10**, 1075–1086. (doi:10.1046/j.1365-294X.2001.01283.x)

Deutsch, J. C. 1997 Colour diversification in Malawi cichlids: evidence for adaptation, reinforcement or sexual selection? *Biol. J. Linn. Soc.* **62**, 1–14. (doi:10.1006/bijl.1997.0135)

Dominey, W. J. 1984 Effects of sexual selection and life history on speciation: species flocks in African cichlids and Hawaiian *Drosophila*. In *Evolution of fish species flocks* (ed. A. A. Echelle & I. Kornfield), pp. 231–250. Orono, Maine: University of Maine at Orono Press.

Egger, B., Meekan, M. G., Salzburger, W., Mwape, L., Makasa, L., Shapola, R. & Sturmbauer, C. 2004 Validation of periodicity of increment formation in otoliths of an African cichlid fish species from Lake Tanganyika, eastern Africa. *J. Fish Biol.* **64**, 1272–1284. (doi:10.1111/j.0022-1112.2004.00390.x)

Fraser, D. J. & Bernatchez, L. 2005 Adaptive migratory divergence among sympatric brook charr populations. *Evolution* **59**, 611–624.

Fryer, G. & Iles, T. D. 1972 *The cichlid fishes of the Great Lakes of Africa: their biology and evolution*. Edinburgh: Oliver & Boyd.

Greenwood, P. H. 1965 The cichlid fishes of Lake Nabugabo, Uganda. *Bull. Br. Mus. Nat. Hist.* **12**, 315–357.

Guo, S. & Thompson, E. 1992 Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics* **48**, 361–372.

Hammnond, H. A., Jin, L., Zhong, Y., Caskey, C. T. & Chakraborty, R. 1994 Evaluation of 13 short tandem repeat loci for use in personal identification applications. *Am. J. Hum. Genet.* **55**, 175–189.

Hendry, A. P. & Day, T. 2005 Population structure attributable to reproductive time: isolation by time and adaptation by time. *Mol. Ecol.* **14**, 901–916. (doi:10.1111/j.1365-294X.2005.02480.x)

Hendry, A. P., Morbey, Y. E., Berg, O. K. & Wenburg, J. K. 2004 Adaptive variation in senescence: reproductive lifespan in a wild salmon population. *Proc. R. Soc. B* **271**, 259–266. (doi:10.1098/rspb.2003.2600)

Knight, M. E. & Turner, G. F. 2004 Laboratory mating trials indicate incipient speciation by sexual selection among populations of the cichlid fish *Pseudotropheus zebra* from Lake Malawi. *Proc. R. Soc. B* **271**, 675–680. (doi:10.1098/rspb.2003.2639)

Knight, M. E., Turner, G. F., Rico, C., van Oppen, M. J. & Hewitt, G. M. 1998 Microsatellite paternity analysis on captive Lake Malawi cichlids supports reproductive isolation by direct mate choice. *Mol. Ecol.* **7**, 1605–1610. (doi:10.1046/j.1365-294x.1998.00478.x)

Kocher, T. D. 2004 Adaptive evolution and explosive speciation: the cichlid fish model. *Nat. Rev. Genet.* **5**, 288–298. (doi:10.1038/nrg1316)

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Paabo, S., Villablanca, F. X. & Wilson, A. C. 1989 Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl Acad. Sci. USA* **86**, 6196–6200.

Kornfield, I. & Smith, P. F. 2000 African cichlid fishes: model systems for evolutionary biology. *Annu. Rev. Ecol. Syst.* **31**, 163–196. (doi:10.1146/annurev.ecolsys.31.1.163)

Kosswig, C. 1947 Selective mating as a factor for speciation in cichlid fish of East African lakes. *Nature* **159**, 604.

Liem, K. F. 1973 Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. *Syst. Zool.* **22**, 425–441.

Manly, B. F. J. 1994 *Multivariate statistical methods. A primer*. Boca Raton, FL: Chapmann & Hall.

Marshall, T. C., Slate, J., Kruuk, L. E. & Pemberton, J. M. 1998 Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **7**, 639–655. (doi:10.1046/j.1365-294x.1998.00374.x)

McKaye, K. R. 1980 Seasonality in habitat selection by the gold colour morph of *Cichlasoma citrinellum* and its relevance to sumpatric speciation in the family Cichlidae. *Environ. Biol. Fishes* **5**, 75–78.

McKaye, K. R. 1991 Sexual selection and the evolution of the cichlid fishes of Lake Malawi, Africa. In *Cichlid fishes: behavior, ecology and evolution* (ed. M. H. A. Keenleyside), pp. 241–257. London: Chapman & Hall.

McKaye, K. R. & Barlow, G. W. 1976 Competition between colour morphs of the midas cichlid, *Cichlasoma citrinellum*, in Lake Jiloa, Nicaragua. In *Investigations of the ichthyofauna of Nicaraguan lakes* (ed. T. B. Thorson), pp. 465–475. Lincoln, Nebraska: School of Life Sciences, University of Nebraska.

McLean, J. E., Bentzen, P. & Quinn, T. P. 2005 Nonrandom, size- and timing-biased breeding in a hatchery population of steelhead trout. *Conserv. Biol.* **19**, 446–454. (doi:10.1111/j.1523-1739.2005.00515.x)

McLennan, D. A. & Ryan, M. J. 1999 Interspecific recognition and discrimination based upon olfactory cues in northern swordtails. *Evolution* **53**, 880–888.

Meyer, A., Kocher, T. D., Basasibwaki, P. & Wilson, A. C. 1990 Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* **347**, 550–553. (doi:10.1038/347550a0)

Østbye, K., Næsje, T. F., Bernatchez, L., Sandlund, O. T. & Hindar, K. 2004 Morphological divergence and origin of sympatric populations of European whitefish (*Coregonus lavaretus* L.) in Lake Femud, Norway. *J. Evol. Biol.* **18**, 683–702. (doi:10.1111/j.1420-9101.2004.00844.x)

Parker, A. & Kornfield, I. 1996 Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Environ. Biol. Fishes* **47**, 345–352. (doi:10.1007/BF00005049)

Pritchard, J. K., Stephens, M. & Donnelly, P. 2000 Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Rice, W. R. 1989 Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Ryan, M. J. & Wagner, W. E. 1987 Asymmetries in mating preferences between species: female swordtails prefer heterospecific males. *Science* **236**, 595–597.

Salzburger, W. & Meyer, A. 2004 The species flocks of East African cichlid fishes: recent advances in molecular phylogenetics and population genetics. *Naturwissenschaften* **91**, 277–290. (doi:10.1007/s00114-004-0528-6)

Salzburger, W., Meyer, A., Baric, S., Verheyen, E. & Sturmbauer, C. 2002 Phylogeny of the Lake Tanganyika cichlid species flock and its relationship to the Central and East African haplochromine cichlid fish faunas. *Syst. Biol.* **51**, 113–135. (doi:10.1080/106351502753475907)

Salzburger, W., Mack, T., Verheyen, E. & Meyer, A. 2005 Out of Tanganyika: genesis, explosive speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. *BMC Evol. Biol.* **5**, 17. (doi:10.1186/1471-2148-5-17)

Schluter, D. 2000 *The ecology of adaptive radiation*. New York: Oxford University Press.

Schneider, S., Roessli, D. & Excoffier, L. 1999 *Arlequin, version 2.0: a software for genetic data analysis*. Geneva: Genetics and Biometry Laboratory, University of Geneva.

Schupke, P. 2003 *Buntbarsche des Tanganjikasees, Teil 1: Die Arten der Gattung Tropheus*. Rodgay, Germany: A.C.S. Verlag.

Seehausen, O. & van Alphen, J. J. M. 1999 Can sympatric speciation by disruptive sexual selection explain rapid evolution of cichlids diversity in Lake Victoria? *Ecol. Lett.* **2**, 262–271. (doi:10.1046/j.1461-0248.1999.00082.x)

Seehausen, O., van Alphen, J. J. M. & Witte, F. 1997 Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* **277**, 1808–1811. (doi:10.1126/science.277.5333.1808)

Seehausen, O., van Alphen, J. J. M. & Lande, R. 1999 Colour polymorphism and sex ratio distortion in a cichlid fish as an incipient stage in sympatric speciation by sexual selection. *Ecol. Lett.* **2**, 367–378. (doi:10.1046/j.1461-0248.1999.00098.x)

Simpson, G. G. 1953 *The major features of evolution*. New York: Columbia University Press.

Slate, J., Marshall, T. & Pemberton, J. 2000 A retrospective assessment of the accuracy of the paternity inference program CERVUS. *Mol. Ecol.* **9**, 801–808. (doi:10.1046/j.1365-294x.2000.00930.x)

Snoeks, J., Rüber, L. & Verheyen, E. 1994 The Tanganyika problem: comments on the taxonomy and distribution patterns of its cichlid fauna. In *Speciation in ancient lakes* (ed. K. Martens, G. Goddeeris & G. W. Coulter), pp. 355–372. Stuttgart: Schweizerbart'sche Verlagsdruckerei.

Stiassny, M. L. J. & Meyer, A. 1999 Cichlids of the Rift Lakes. *Sci. Am.* **280**, 64–69.

Sturmbauer, C. 1998 Explosive speciation in cichlid fishes of the African Great Lakes: a dynamic model of adaptive radiation. *J. Fish Biol.* **53**(Suppl. A), 18–36.

Sturmbauer, C. & Dallinger, R. 1995 Diurnal variation of spacing and foraging behavior in *Tropheus moorii* (Cichlidae) in Lake Tanganyika. *Neth. J. Zool.* **45**, 386–401.

Sturmbauer, C. & Meyer, A. 1992 Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. *Nature* **358**, 578–581. (doi:10.1038/358578a0)

Sturmbauer, C., Baric, S., Salzburger, W., Rüber, L. & Verheyen, E. 2001 Lake level fluctuations synchronize genetic divergence of cichlid fishes in African lakes. *Mol. Biol. Evol.* **18**, 144–154.

Sturmbauer, C., Koblmuller, S., Sefc, K. M. & Duftner, N. 2005 Phylogeographic history of the genus *Tropheus*, a lineage of rock-dwelling cichlid fishes endemic to Lake Tanganyika. *Hydrobiologia* **542**, 335–366. (doi:10.1007/s10750-004-4664-y)

Swofford, D. L. 2002 *PAUP*—phylogenetic analyses using parsinomy and other methods, version 4.0*. Sunderland, MA: Sinauer.

Turner, G. F. & Burrows, M. T. 1995 A model of sympatric speciation by sexual selection. *Proc. R. Soc. B* **260**, 287–292.

van Oppen, M. J., Rico, C., Deutsch, J. C., Turner, G. F. & Hewitt, G. M. 1997 Isolation and characterization of microsatellite loci in the cichlid fish *Pseudotropheus zebra*. *Mol. Ecol.* **6**, 387–388. (doi:10.1046/j.1365-294X.1997.00188.x)

van Oppen, M. J., Turner, G. F., Rico, C., Robinson, R. L., Deutsch, J. C., Genner, M. J. & Hewitt, G. M. 1998 Assortative mating among rock-dwelling cichlid fishes supports high estimates of species richness from Lake Malawi. *Mol. Ecol.* **7**, 991–1001. (doi:10.1046/j.1365-294x.1998.00417.x)

Verheyen, E., Salzburger, W., Snoeks, J. & Meyer, A. 2003 Origin of the superflock of cichlid fishes from Lake Victoria, East Africa. *Science* **300**, 325–329. (doi:10.1126/science.1080699)

Weir, B. S. & Cockerham, C. C. 1984 Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.

Zardoya, R., Vollmer, D. M., Craddock, C., Streelman, J. T., Karl, S. & Meyer, A. 1996 Evolutionary conservation of microsatellite flanking regions and their use in resolving the phylogeny of cichlid fishes (Pisces: Perciformes). *Proc. R. Soc. B* **263**, 1589–1598.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.