

Recent speciation between sympatric Tanganyikan cichlid colour morphs

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Abstract

Lake Tanganyika, Africa's oldest lake, harbours an impressive diversity of cichlid fishes. Although diversification in its radiating groups is thought to have been initially rapid, cichlids from Lake Tanganyika show little evidence for ongoing speciation. In contrast, examples of recent divergence among sympatric colour morphs are well known in haplochromine cichlids from Lakes Malawi and Victoria. Here, we report genetic evidence for recent divergence between two sympatric Tanganyikan cichlid colour morphs. These *Petrochromis* morphs share mitochondrial haplotypes, yet microsatellite loci reveal that their sympatric populations form distinct genetic groups. Nuclear divergence between the two morphs is equivalent to that which arises geographically within one of the morphs over short distances and is substantially smaller than that among other sympatric species in this genus. These patterns suggest that these morphs diverged only recently, yet that barriers to gene flow exist which prevent extensive admixture despite their sympatric distribution. The morphs studied here provide an unusual example of active diversification in Lake Tanganyika's generally ancient cichlid fauna and enable comparisons of speciation processes between Lake Tanganyika and other African lakes.

Keywords: assortative mating, cichlid, colour morph, Lake Tanganyika, speciation

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Introduction

The cichlid fish radiations of East Africa are hailed as the most diverse vertebrate species flocks (Kocher 2004) and as the animal group with the fastest-known speciation rates (McCune 1997). Less appreciated, however, are the dramatic differences among cichlid radiations in different African lakes, among which Lake Tanganyika is an outlier owing to its extremely old age (9–12 Myr; Cohen *et al.* 1993). Lakes Malawi and Victoria are much younger: the Malawi basin dates to 5 Myr (Delvaux 1995) and may have been uninhabitable by cichlids as

recently as 90 000 kya because of megadrought conditions (Cohen *et al.* 2007), and Lake Victoria was dry 15 000 kya (Johnson *et al.* 1996; Stager & Johnson 2008). In contrast with the single-lineage origins of cichlid diversity in Lakes Malawi and Victoria (but see Joyce *et al.* 2010; Seehausen *et al.* 2003), the Tanganyikan cichlid fauna descends from eight colonizing lineages (Salzburger *et al.* 2002, 2005). Species richness in Lake Tanganyika is substantially less than in Lakes Malawi and Victoria (Tanganyika: ~250 spp.; Malawi: 451–600 spp.; Victoria: 447–535 spp.; Genner *et al.* 2004), and diversification rates in Tanganyika are much lower than in the recent, rapid radiations of Malawi and Victoria (Day *et al.* 2008). Lake Tanganyika is considered the ancestral source of the riverine lineages that seeded the haplochromine radiations of both Malawi and Victoria (Salzburger *et al.* 2005). However, unlike these radiations, where sharing of polymorphism among species is rampant because of extremely recent divergence and ongoing hybridization (e.g. Meyer *et al.* 1990; Seehausen

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et al. 1997; Nagl *et al.* 1998; Won *et al.* 2006), Lake Tanganyika's cichlid groups are older clades in which no cases of incomplete lineage sorting of mitochondrial DNA at the species level have been described (Sturmbauer *et al.* 2003).

The existence of sympatric, closely related colour morphs in Lakes Malawi and Victoria has spurred interest in these fishes as potential cases of sympatric speciation (Seehausen & van Alphen 1999). In contrast, although geographic differences in colour are common in Tanganyikan cichlids (Kohda *et al.* 1996) and are well-studied in the genus *Tropheus* (Baric *et al.* 2003; Egger *et al.* 2007; Koblmüller *et al.* 2011), sympatric colour morphs are extremely rare in Lake Tanganyika (for a well-studied case of artificial sympatry, see Salzburger *et al.* 2006). Tanganyika's cichlids thus represent an important contrast to the haplochromines of Lakes Malawi and Victoria. Did cichlid speciation mechanisms differ in Lake Tanganyika compared to the younger radiations of Lakes Malawi and Victoria? Or do the differences in diversity arise from speciation-independent mechanisms, like the winnowing of ecologically similar forms through extinction after speciation? Understanding how processes of speciation in Tanganyika's cichlids compare and contrast with those of other lakes will provide insight into the interplay between diversity-generating mechanisms and long-term ecological processes in determining patterns of extant diversity in African cichlids and provide general insight into the processes influencing the generation and maintenance of biodiversity.

The following are the crucial questions for understanding speciation in sexually reproducing organisms, under any geographic scenario: (i) What maintains reproductive isolation between recently separated species? (ii) How did these isolating mechanisms originate? Although full tests of these questions in wild populations are exceedingly difficult, our best approaches involve careful study of very recently diverged species and of populations in the process of divergence (Coyne & Orr 2004; Maan *et al.* 2004; Seehausen *et al.* 2008). Situations where close relatives are found in natural sympatry present a valuable opportunity to examine the forces operating to create and maintain reproductive isolation between recently diverged species, but such systems have not previously been investigated in Lake Tanganyikan cichlids because of the rare occurrence of sympatrically distributed sister taxa. Here, we describe a case of sympatric coexistence of colour morphs of cichlids from the genus *Petrochromis* in the region of Kigoma, Tanzania. We ask whether the colour variation we observe in this region represents a case of colour polymorphism segregating within panmictic populations, or whether there is evidence for genetic diver-

gence and the origin of reproductive barriers between the colour morphs.

Study system

Petrochromis cichlids are members of the tribe Tropheini, the Tanganyikan lineage that is sister to the clade that includes the haplochromine radiations of Lakes Malawi and Victoria. Like cichlids from the radiations in Malawi and Victoria, tropheines maternally mouthbrood their young and are generally polygamous (Brichard 1989). However, other characteristics of the tropheine radiation contrast substantially with the Malawi and Victoria haplochromine radiations. Tropheine cichlids are not nearly as diverse (24 spp; Koblmüller *et al.* 2010), nor do they show the dramatic sexual dichromatism and colour polymorphism common in haplochromines from Lakes Malawi and Victoria (Seehausen *et al.* 1999a; Dijkstra *et al.* 2009; Roberts *et al.* 2009).

In the Kigoma region of Lake Tanganyika, up to six distinct forms of *Petrochromis* are found in sympatry. Two of these are described species known to be genetically distinct (*Petrochromis orthognathus*, Matthes 1959; *Petrochromis famula*, Matthes & Trewavas 1960). The other four are undescribed forms with varying degrees of phenotypic distinctiveness (*P.* sp. 'kazumbe', *P.* sp. 'moshi', *P.* sp. 'green', *P. cf. polyodon*; see Table S1 [Supporting information] for the origin and history of these names). The phylogenetic relationships among these sympatric morphs/species have not previously been investigated, and because the species status of the undescribed forms is not well known, we will refer to all of them here as 'morphs'.

In previous work, we demonstrated that *P.* sp. 'kazumbe' and sympatric *P.* sp. 'moshi', both well-recognized morphs in the aquarium trade, have strongly and congruently geographically structured populations in the Kigoma region of Lake Tanganyika (Wagner & McCune 2009). Specifically, we showed that genetic breaks in *P.* sp. 'kazumbe' and *P.* sp. 'moshi' populations coincide with two long stretches of sandy habitat, the Kigoma Bay and the Luiche River delta, subdividing these species into northern, mid and southern genetic groups within the region (Wagner & McCune 2009). Another *Petrochromis* morph, *Petrochromis cf. polyodon* (Boulenger 1898), occurs in sympatry with *P.* sp. 'kazumbe' and *P.* sp. 'moshi' in the Kigoma region. Adults of *P.* sp. 'kazumbe' and *P. cf. polyodon* are much more phenotypically similar than are any other two *Petrochromis* species/morphs in the Kigoma region, but they are readily distinguishable based on the amount of orange coloration on the body, head and fins: *P.* sp. 'kazumbe' has a mostly orange coloration with a light grey background, whereas *P. cf. polyodon* is predominantly

light grey-blue with a limited amount of orange coloration. Given the sympatric distributions of *P. cf. polyodon* and *P. sp. 'kazumbe'*, and the importance of coloration in other cichlid groups as a cue for assortative mating, we here investigate whether these colour morphs represent polymorphism within panmictic populations, or whether there is population genetic evidence for assortative mating within these sympatric morphs, suggesting that they are incipient species. Using mtDNA sequence data and multi-locus microsatellite genotypes, we tested for genetic differentiation between these morphs via haplotype analyses and Bayesian analyses of population structure. To provide a comparative scale for the small magnitude of genetic differentiation between these two morphs, we conducted phylogenetic analyses based on sequence data collected from all tropheine cichlids present in the Kigoma region, and population genetic analyses based on microsatellite data from their close *Petrochromis* relatives.

Materials and methods

Sample collection

We collected *P. sp. 'kazumbe'* (hereafter the 'orange morph') at eight sites spanning 60 km of coastline in the Kigoma region of Tanzania in 2005 and 2007 (Table S1, Supporting information). We also collected individuals that we identified as *Petrochromis cf. polyodon* (hereafter the 'blue morph') at four of these sites on the same visits. We collected no juveniles (<10 cm standard length) with blue morph coloration; all juveniles collected had orange morph coloration. We collected fish using gill nets while snorkelling in the rocky littoral zone between 1 and 10 m in depth. In this depth range, orange morph *Petrochromis* are far more common than are blue morph *Petrochromis* (C. Wagner, unpublished data); their relative abundance at greater depths is unknown. There is no obvious microhabitat or depth segregation between the morphs and they are often seen swimming side by side (C. Wagner, personal observation). As a metric of genetic divergence among other sympatric close relatives, we analysed samples of the four other *Petrochromis* species that are found sympatrically with the orange and blue morph *Petrochromis*. These included *P. orthognathus*, *P. famula* and the undescribed species *P. sp. 'moshi'* and *P. sp. 'green'*. Additionally, representatives from all other tropheine cichlid species found in the Kigoma region (nine species in seven genera) were collected at a subset of these sites in 2002, 2005 and 2007. Fin clips were preserved in DMSO-EDTA buffer (Seutin & White 1991) for genetic work. All specimens were retained as vouchers and

have been deposited in the Cornell Museum of Vertebrates (Table S1, Supporting information).

DNA extraction, PCR, sequencing, genotyping

We extracted genomic DNA from fin clips using DNeasy Tissue Kits (Qiagen). We PCR-amplified and sequenced the mitochondrial nitrogen dehydrogenase subunit 2 gene (ND2, 1047 bp) from a subset of our total samples of both colour morphs, using the primers and conditions described in Wagner *et al.* (2009). We also sequenced ND2 and the mitochondrial cytochrome B oxidase gene (cytB, 1149 bp) from both colour morphs and all other tropheine cichlid species and morphs found in the Kigoma region, using methods described in Wagner *et al.* (2009).

To assess genetic differentiation between colour morphs, we amplified 11 microsatellite loci previously developed for Tanganyikan and Malawian cichlid species (Table S2, Supporting information) for 217 orange morph individuals and 24 blue morph individuals (see Table S2, Supporting information). To provide a comparative scale, we genotyped individuals of the other two sympatric undescribed *Petrochromis* species, *P. sp. 'moshi'* (see also Wagner & McCune 2009) and *P. sp. 'green'*, using the same loci. Details of the PCR and genotyping reactions are described in Wagner & McCune (2009). We scored microsatellite genotypes using Genemapper version 3.7 (Applied Biosystems) and verified each genotype by eye. For the orange and blue morph *Petrochromis*, all samples were genotyped and scored blind to the field species identification of the sample, and all scoring used the same panels and bin sets for all loci and all individuals.

Data analysis

To place the relationship of the orange and blue *Petrochromis* morphs in a broader phylogenetic context, we conducted a phylogenetic analysis using Mr. Bayes 3.1.2 (Huelsenbeck *et al.* 2001; Ronquist & Huelsenbeck 2003) of ND2 and cytB sequences representing the full Kigoma region tropheine cichlid community. We ran Mr. Bayes for two runs of 10 million generations each and discarded the first 10% of trees to account for burn-in, leaving the posterior distribution of trees with standard deviations of split frequencies below 0.01. We then used the additional ND2 sequences we collected from orange and blue morph individuals, and haplotype networks created using the computer program TCS (Clement *et al.* 2000), to explore mitochondrial genetic variation between these two morphs.

Using microsatellite genotypes, we employed the Bayesian clustering program STRUCTURE v.3.3 (Pritchard

et al. 2000) to find the most likely number of genetic groups (K) for the complete set of orange and blue morph individuals. We ran STRUCTURE for values of K from 1 through 10, for 10 runs at each K value. Each run consisted of 180 000 generations of burn-in and 1 million generations post-burn-in, used the admixture and correlated allele frequency models, and were set to infer the parameter alpha separately for each population. We then assessed the most likely number of genetic groups by examining the value of K for which $\text{LnP}(D)$ values reached their maximum (Pritchard & Wen 2003). We used the program CLUMPP (Jakobsson & Rosenberg 2007) to summarize results over multiple runs and *Distruct* (Rosenberg 2004) to plot STRUCTURE results for the most likely K value.

Using microsatellite data, we calculated F_{ST} values for all pairs of population samples identified through STRUCTURE analyses. We also calculated pairwise differences between *P. sp.* 'moshi', *P. sp.* 'green' and the orange and blue morph groups. We used Weir & Cockerham's (1984) estimator and tested for significance using permutation tests of 10 000 replicates in GENETIX 4.05.2 (Belkhir *et al.* 1996–2004). To evaluate the effects of individual loci on estimates of F_{ST} , we performed jackknife analyses over loci, as described in Wagner & McCune (2009).

Results

Phylogenetic analyses of mtDNA sequences for the trophaine cichlid communities sampled in this study support the grouping of four *Petrochromis* morphs found in the Kigoma region: *P. sp.* 'green', *P. sp.* 'moshi', and the orange and blue *Petrochromis* morphs (Fig. 1a). The orange and blue morphs cannot be distinguished based

on mtDNA sequence data. As expected of well-differentiated species, individuals of *P. sp.* 'green' and *P. sp.* 'moshi' each form strongly supported monophyletic groups, whereas the orange and blue morph *Petrochromis* individuals together form a strongly supported but intermixed haplotype clade (Fig. 1b). This haplotype mixing contrasts with the 11 additional trophaine species in the analysis, all of which are represented by multiple sequences and show strong evidence for reciprocal monophyly with respect to all other species sampled (Fig. S1, Supporting information). Phylogenetic resolution of basal relationships in the tree is generally poor (Fig. S1, Supporting information), as has been shown in other phylogenetic studies of trophaine cichlids and interpreted as an indication of ancient rapid radiation and hybridization (Sturmbauer *et al.* 2003; Koblmüller *et al.* 2010).

Eleven ND2 haplotypes were recovered from a subsample of 36 orange and blue morph individuals. Twenty-two of these individuals (both orange and blue morph) shared one common haplotype, three other haplotypes were shared between morphs and the only haplotypes not shared among morphs were haplotypes recovered from single individuals (Fig. 1b). All sequences have been deposited in GenBank (Table S1, Supporting information).

STRUCTURE runs supported four genetic groups among orange and blue morph genotypes (Fig. 2; Fig. S2, Supporting information). Three of these represent spatial genetic structure from the extensively sampled orange morph populations, forming 'north', 'mid' and 'south' genetic groups ($n = 130$, $n = 59$ and $n = 28$, respectively), as described more fully in Wagner & McCune (2009). The fourth genetic group ($n = 24$) includes all fish that were identified as the blue morph in the field

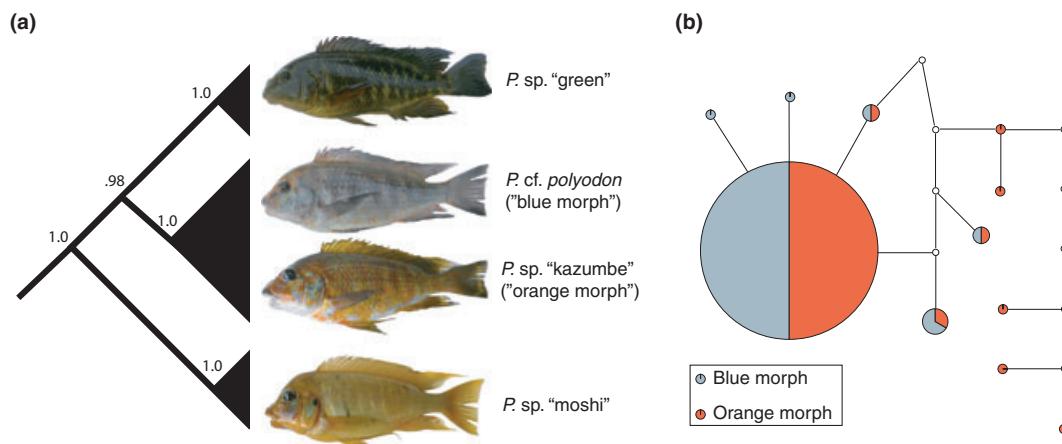


Fig. 1 (a) Two undescribed sympatric *Petrochromis* species plus the orange and blue *Petrochromis* morphs form a strongly supported mitochondrial clade. Whereas *P. sp.* 'green' and *P. sp.* 'moshi' are reciprocally monophyletic, the orange and blue *Petrochromis* morphs share mitochondrial haplotypes. (b) Relationships among ND2 haplotypes recovered from orange and blue morph *Petrochromis* individuals. Circle sizes are proportional to the number of individuals with a given haplotype.

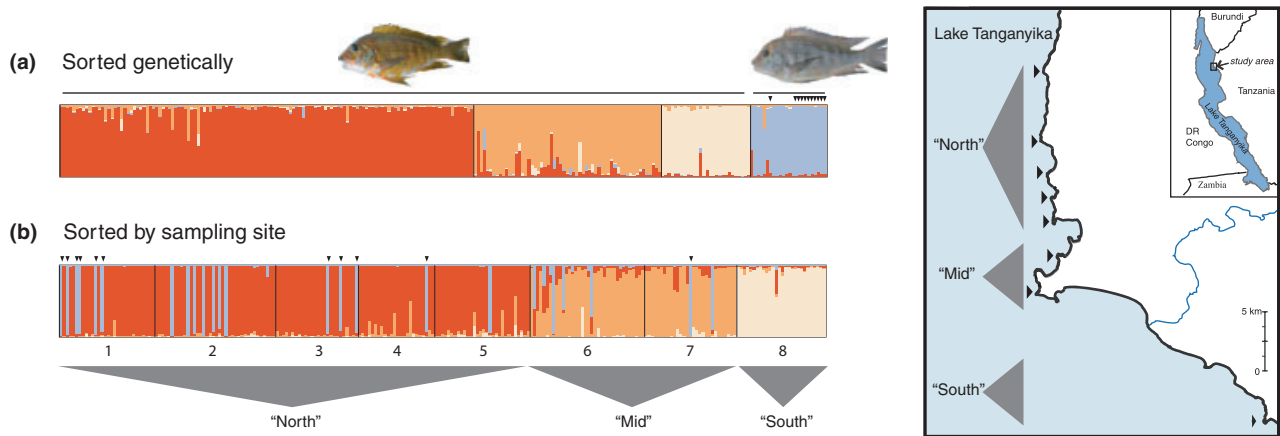


Fig. 2 STRUCTURE analyses support the existence of four genetic groups ($K = 4$) among individuals from eight sampling sites. Three of these groups represent geographic divergence within the orange morph; the fourth is blue morph individuals found in sympatry with orange morph individuals. This demonstrates that orange morph and blue morph populations are not panmictic, despite their sympatric distribution. In (a) individuals are sorted by genetic group; in (b) individuals are sorted by sampling site (1–8). Within the orange morph, there are three geographically distributed groups, 'north', 'mid' and 'south' (corresponding to the three shades of orange; $n = 130$, $n = 59$ and $n = 28$, respectively), as reported in Wagner & McCune (2009). Blue morph individuals (blue), regardless of sampling location, form the fourth genetic group ($n = 24$). Black triangles correspond to individuals identified in the field as the blue morph. Individual assignment probabilities shown here are the consensus generated by CLUMPP from 10 STRUCTURE runs of $K = 4$.

($n = 11$), all of which were adults >10 cm in standard length. All of these fish had assignment probabilities $>95\%$ to this fourth genetic group. This fourth genetic group additionally includes juvenile fish (<10 cm) not recognized as the blue morph in the field because of their orange juvenile coloration ($n = 13$). Three juvenile fish in total assigned to the fourth genetic group had assignment probabilities $<88\%$ (60, 62 and 72%). One fish was split 38, 33 and 28% into the first, second and fourth genetic groups, respectively.

All pairwise F_{ST} s between STRUCTURE-assigned populations were highly significant. Pairwise F_{ST} values were highest in comparisons of the blue morph with the orange morph geographic subset populations (Fig. S3, Supporting information). The pairwise F_{ST} value for the northernmost versus southernmost orange morph populations (Wagner & McCune 2009) is nearly equivalent to the pairwise F_{ST} value for pooled orange morph geographic populations versus the blue morph (0.19 vs. 0.21; Fig. 3). All other pairwise F_{ST} values between morphs are substantially higher (>0.3) than the value for orange morph versus blue morph individuals (Fig. 3).

Discussion

We found substantial genetic evidence for very recent divergence between two sympatric cichlid colour morphs in Lake Tanganyika. In STRUCTURE analyses, all individuals identified in the field as the blue morph are unambiguously assigned to a genetic group separate

from sympatric orange morph individuals. This result implies that mechanisms inhibiting extensive hybridization between the orange and blue *Petrochromis* morphs have evolved, allowing their differentiation to be maintained in sympatry, and indicating that they should be considered incipient species. Pairwise F_{ST} values between the blue morph group and the orange morph group are equivalent in magnitude to those between orange morph populations separated by only 60 km of shoreline, suggesting that the divergence between these sympatric morphs occurred recently. Other comparable pairwise divergences for closely related sympatric *Petrochromis* morphs are substantially higher than that between the orange and blue morphs (Fig. 3). This genetic signature of recent speciation has not been previously described in Lake Tanganyikan cichlids and is unexpected given that these fish lack the hallmark sexual dimorphism and striking colour polymorphisms present in rapidly and recently diversifying haplochromine cichlid lineages (Seehausen *et al.* 1999b).

Recent divergence and evidence for the evolution of barriers to gene flow

Previous phylogenetic work has suggested that reciprocal monophyly of Tanganyikan cichlid species at mtDNA loci is effectively universal (Sturmbauer *et al.* 2003), in strong contrast to extensive sharing of mitochondrial haplotypes among cichlid species in Lakes Malawi and Victoria (e.g. Meyer *et al.* 1990; Nagl *et al.* 1998; Won *et al.* 2006). Previously documented cases of

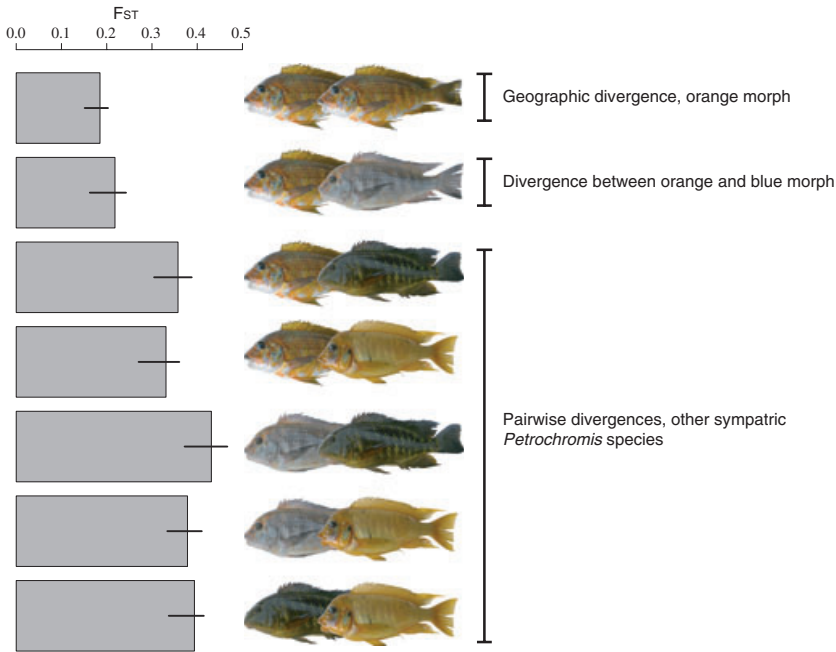


Fig. 3. Nuclear divergence between the orange and blue *Petrochromis* morphs is of equivalent magnitude to geographic divergence within the orange morph and substantially smaller than divergence between other sympatric *Petrochromis* morphs. This figure depicts pairwise F_{ST} values for the northernmost orange morph population with the southernmost (top), the value for pooled orange morph populations vs. blue morph (second from top) and all other pairwise combinations of the orange and blue morphs with *P. sp.* 'moshi' and *P. sp.* 'green'. Black error bars represent minimum and maximum F_{ST} values from jackknifing over loci.

mtDNA haplotype sharing among Tanganyikan species have been interpreted as evidence for hybridization and not as evidence for recent divergence and incomplete lineage sorting (e.g. Ruber *et al.* 2001; Koblmüller *et al.* 2007; Nevado *et al.* 2011). Unlike the Tanganyikan norm, we find extensive mitochondrial haplotype sharing between the orange and blue *Petrochromis* morphs of this study (Fig. 1b). This lies in distinct contrast to comparisons of the other sympatric *Petrochromis* species found in the Kigoma region, where sequence divergence between species is 2–4%, and all sequenced individuals are reciprocally monophyletic at the species level (Fig. 1a and Fig. S1, Supporting information).

Despite their extensive sharing of mtDNA haplotypes, we find strong genotypic support for the hypothesis that the sympatric orange morph and blue morph groups are separately breeding populations. Bayesian analysis of microsatellite variation assigns all fish identified in the field as the blue morph to a single genetic group, with assignments >95% in all cases (Fig. 2). These fish were collected at the same field sites and in the same nets as orange morph individuals collected from both 'north' and 'mid' spatial genetic groups. Therefore, regardless of their geographic origin, blue morph individuals are more closely related to each other at nuclear loci than they are to orange morph individuals. This genetic pattern provides strong evidence that barriers to gene flow between the morphs have evolved, allowing them to remain distinct genetic groups in sympatry.

The existence of extensive sharing of mitochondrial haplotypes could be the result of (i) introgression associated with recent and/or ongoing hybridization, (ii)

recent divergence and incomplete lineage sorting at mitochondrial loci, or a combination of these processes. We examine each of these scenarios below.

Hybridization could produce sharing of mitochondrial haplotypes between orange and blue morph *Petrochromis*. However, assignment probabilities of >88% for the 20 of the 24 fish assigned to the blue morph genetic group suggest that ongoing hybridization is not extensive. The few fish that have admixed genetic backgrounds based on STRUCTURE assignment probabilities could be backcrossed hybrid individuals, but this pattern could also result from sharing of ancestral polymorphism. If hybridization were extensive, many more individuals with admixed genetic backgrounds would be expected.

Because extensive ongoing hybridization would not maintain the clear nuclear differentiation that we observe, we interpret the shared mitochondrial haplotypes between orange and blue morphs as most likely the product of incomplete lineage sorting resulting from the recency of the divergence event creating these morphs. Our data do not exclude the possibility of low levels of ongoing hybridization, but they support a substantial degree of reproductive isolation between the morphs, despite their sympatric distribution. This evidence for reproductive isolation in sympatry suggests that we can consider these morphs to be incipient species.

Speciation scenarios

Given that these *Petrochromis* morphs are currently in sympatry and that their divergence is recent, it is possible that they diverged in sympatry. However, the

current sympatric distribution of these fishes does not necessarily imply sympatric divergence, as allo- or parapatric divergence, followed by a return to sympatry, is also possible. *Petrochromis* species have extremely fine-scale spatial genetic structuring, and extensive sandy habitats appear to inhibit gene flow between populations (Wagner & McCune 2009). Additionally, many of the recognized colour variants in this and other closely related genera are geographically separated within Lake Tanganyika, implying that geographic divergence in colour is common in these fishes (Kohda *et al.* 1996; Baric *et al.* 2003). Lake-level fluctuations, which have occurred repeatedly in Lake Tanganyika, could have brought geographically distinct populations into sympatry, providing a mechanism for secondary contact between colour-divergent populations (Koblmüller *et al.* 2011). Large-scale geographic surveys of the distribution of these species would help to assess the possibility of secondary sympatry in the Kigoma region, as their distribution outside the 60 km of coastline surveyed in this study is unknown.

That said, if sympatry between the orange and blue morphs is attributed to secondary contact, either geographic divergence must have been on small geographic scales or the morphs underwent a period of hybridization upon secondary contact that decreased their differentiation at nuclear loci. Even short geographic distances separating populations of the orange morph result in divergences of equivalent magnitude to that observed between orange and blue morphs. The scale of geographic divergence in the orange morph does not appear to be unusual for the genus, as *P. sp.* 'moshi' exhibits geographic divergence in the same region that is equivalent in magnitude to that of the orange *Petrochromis* morph (Wagner & McCune 2009).

Regardless of the geography of speciation in this scenario, the current sympatric distribution of these *Petrochromis* morphs implies that barriers to gene flow have evolved and that these barriers act to inhibit extensive gene flow between them. Evidence from studies of hybrid viability suggests that pre-mating isolating barriers are far more important to speciation in other cichlid species than are post-mating barriers (Stelkens *et al.* 2010). Numerous studies of Lake Malawi and Lake Victoria cichlids support a role for male colour in assortative mating (e.g. Seehausen & van Alphen 1998; Allender *et al.* 2003; Knight & Turner 2004). Three studies using the Tanganyikan trophaine genus *Tropheus* also provide some evidence for assortative mating among colour morphs based on coloration (Salzburger *et al.* 2006; Egger *et al.* 2008, 2010). At a few sites, multiple *Tropheus* morphs co-occur, and in some cases hybridize extensively (e.g. at the site Katoto; Egger *et al.* 2007), and in other cases appear to remain distinct (e.g.

at the site Ikola, and where the morph 'Kirschfleck' co-occurs with other species; Egger *et al.* 2007). However, the mechanisms maintaining the morphs, or leading to their extensive hybridization, have not been studied in the field. Given the common role for coloration in assortative mating in cichlids, it is reasonable to suggest that coloration may play an important role in mate choice in the *Petrochromis* morphs studied here.

A further suggestion that colour may be important in assortative mating in this system is that the colour differences between these morphs apparently arise late in ontogeny. We categorized all individuals <10 cm in standard length as juveniles, and in this size class, all individuals expressed orange coloration. However, many of these orange juveniles are members of the blue morph genetic group (Fig. 2). This suggests that juveniles of the orange and blue morphs are not distinct in colour and that the colour distinction between the morphs arises later in ontogeny. Late ontogeny colour shifts have also been described in Lake Victoria cichlids (Maan *et al.* 2006) and in Neotropical Midas cichlids; in the latter case, colour morphs show evidence for assortative mating (Barlow 1986; Barlow *et al.* 1990; Elmer *et al.* 2009). A correlation between the timing of sexual maturity and the time at which colour differences arise in ontogeny suggests a role for colour in sexual selection.

Sympatric divergence of the orange and blue *Petrochromis* morphs is also a possibility, given their relative magnitudes of genetic divergence and their current sympatric distribution. Both theoretical and empirical work show that interactions between ecology and sexual selection may have important impacts on the mechanisms whereby new species arise (Ritchie 2007; Maan & Seehausen 2011), and these linkages may be particularly important in sympatric speciation. Recent work on Lake Victoria cichlids provides support for a 'sensory drive' mechanism for speciation, where the light environment, which changes as a function of water depth and clarity, provides a basis for (i) natural selection for environment-specific visual acuity and (ii) sexual selection for male sexual signals, which results in speciation (Kawata *et al.* 2007; Seehausen *et al.* 2008; Maan & Seehausen 2010). This is an intriguing model to consider in the context of cichlid speciation in other environments. However, Lake Tanganyika's littoral light environment differs substantially from that of Lake Victoria, and it remains unknown whether cichlid speciation via similar mechanisms could occur in this setting.

Ecological factors other than depth could also influence the maintenance of diversity and/or speciation in this group. Environmental heterogeneity contributes to the maintenance of colour polymorphism in many animal systems (e.g. Rosenblum 2006; Gray *et al.* 2008), and if this heterogeneity is stable over time, it can lead

to niche partitioning (Endler & Thery 1996; Leal & Fleishman 2002) and in some circumstances could promote speciation (Chunco *et al.* 2007; Gray & McKinnon 2007). Although studies of microhabitat partitioning are lacking for *Petrochromis*, the rocky environments that constitute *Petrochromis* habitat are complex, and different microhabitats might offer consistent differences in light environment that could facilitate natural and sexual selection via sensory drive in a manner analogous to depth gradients. Alternatively, assortative mating could result by habitat sorting alone if colour was associated with microhabitat preference, and it could thereby influence the maintenance and/or the origins of diversity. Whether ecological differences arise during or after the process of speciation and how ecology and sexual signals interact during and after speciation are compelling and open questions in cichlid diversification and in adaptive radiation in general.

In conclusion, genetic evidence suggests that speciation in the *Petrochromis* morphs studied here is more recent than in previously studied sympatric cichlid species in Lake Tanganyika. Although *Petrochromis* and other tropheine cichlids lack the hallmark sexual dichromatism and colour polymorphisms present in rapidly diversifying haplochromine cichlid lineages, it is perhaps not a coincidence that we find recent speciation in the Lake Tanganyikan group that is sister to the haplochromines. Comparative studies are key to understanding the origins and maintenance of diversity in ecologically complex and hyperdiverse systems. Understanding the ecological context for speciation in this Tanganyikan system will provide context for studies of cichlid speciation on similarly recent timescales in the younger lakes that have produced such spectacular examples of vertebrate diversity.

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This study is part of work done by C.E.W. for her PhD dissertation on population genetics, speciation and macroevolutionary patterns in African cichlid fishes. A.R.M. supervised C.E.W.'s PhD work and does research focusing on the speciation, macroevolution and evolutionary developmental biology of fishes. Laboratory work for this project was conducted through the Fuller Evolutionary Biology Program at the Cornell Lab of Ornithology, which is directed by I.J.L. I.J.L.'s research uses phylogenetic and population genetic tools to investigate the origins and maintenance of diversity in animal systems.

Data accessibility

DNA sequences: GenBank accession numbers are listed in online supporting Table S1 (Supporting information).

Microsatellite data: DRYAD doi: 10.5061/dryad.t6s441n0.

Supporting information

Fig. S1 Phylogenetic relationships among tropheine cichlids from the Kigoma region based on *cytB* and *ND2* sequences.

Fig. S2 $LnP(D)$ values from *STRUCTURE* over 10 runs each at 10 values of K .

Fig. S3 Pairwise F_{ST} values from populations identified in *STRUCTURE* runs.

Table S1 Species studied and specimen information.

Table S2 Allelic diversity and size range for the 11 microsatellite loci used in this study.

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