Monogamy in the maternally mouthbrooding Lake Tanganyika cichlid fish *Tropheus moorii*

Bernd Egger¹, Beate Obermüller¹, Harris Phiri², Christian Sturmbauer¹
and Kristina M. Sefc^{1,*}

¹Department of Zoology, University of Graz, Universitätsplatz 2, 8010 Graz, Austria

²Fisheries Research Division, Department of Research and Specialist Services, Ministry of Agriculture,
Food and Fisheries, PO Box 55, Mpulungu, Zambia

Supported by evidence for assortative mating and polygynandry, sexual selection through mate choice was suggested as the main force driving the evolution of colour diversity of haplochromine cichlids in Lakes Malawi and Victoria. The phylogenetically closely related tribe Tropheini of Lake Tanganyika includes the genus *Tropheus*, which comprises over 100 colour variants currently classified into six morphologically similar, polyphyletic species. To assess the potential for sexual selection in this sexually monochromatic maternal mouthbrooder, we used microsatellite-based paternity inference to investigate the mating system of *Tropheus moorii*. In contrast to haplochromines in Lake Malawi, multiple paternity is rare or even absent in broods of *T. moorii*. Eighteen of the 19 analysed families were consistent with genetic monogamy, while either a mutation or more than one sire explained the genotype of one offspring in another brood. We discuss the differences in breeding behaviour between *T. moorii* and the Lake Malawi haplochromines, and evaluate additional factors or alternatives to sexual selection as promoters of colour diversification. A preliminary survey of other Tropheini species suggested that multiple paternity is infrequent in the entire tribe.

Keywords: mating system; paternity; sexual selection; colour diversification; social competition

1. INTRODUCTION

The cichlid fishes in the East African Great Lakes embrace a broad diversity of mating systems, breeding behaviours and forms of parental care (Fryer & Iles 1972; Barlow 1991; Kuwamura 1997). So far, the study of cichlid mating behaviour has relied mainly on field and laboratory observations, and molecular methods for parentage analysis have been applied to a few selected species only (Kellogg *et al.* 1995, 1998; Parker & Kornfield 1996; Dierkes *et al.* 1999; Taylor *et al.* 2003; Maan *et al.* 2004; Knight & Turner 2004; Katoh *et al.* 2005). Numerous examples from various animal groups, notably birds, mammals and fish, illustrate the shortcomings of observational characterization of mating systems due to the inaccessibility of the mating location for the observer and discrepancies between social and genetic mating systems (the true parentage as opposed to the apparent social interaction), e.g. through sneaking and cuckoldry (e.g. Hughes 1998; Avise *et al.* 2002; Griffiths *et al.* 2002). However, it is the genetic rather than the social mating system that has ramifications for several evolutionarily important parameters. Modes and rates of speciation, a topic of particular interest in the rapidly radiating lacustrine Cichlidae, are influenced by mating behaviour through effective population size (Sugg & Chesser 1994), reproductive bias in one or both sexes (Jones *et al.* 2001;

Avise *et al.* 2002), with consequences for sexual selection (West-Eberhard 1983; Dominey 1984; Panhuis *et al.* 2001) and rates of gene flow and assortative mating (Kondrashov & Shpak 1998; Van Oppen *et al.* 1998). Indeed, one explanation for the enormous species richness and variety in secondary sexual characters of haplochromine cichlids in Lake Malawi and Lake Victoria is that sexual selection, associated with polygynous mating systems, drove diversification of spatially segregated (e.g. Danley & Kocher 2001; Knight & Turner 2004; Pauers *et al.* 2004) or even of sympatric populations (e.g. Seehausen *et al.* 1998; Seehausen & van Alphen 1999; Maan *et al.* 2004; but see Arnegard & Kondrashov 2004; Coyne & Orr 2004). In contrast, the morphological and behavioural diversity of the lineages of Lake Tanganyika cichlids was largely attributed to natural selection on allopatric populations occupying different ecological niches (Sturmbauer 1998).

Many of the Lake Tanganyika species display geographical variation in coloration (Konings 1998), most strikingly realized in the over 100 colour variants within the genus *Tropheus* (Schupke 2003), a member of the endemic tribe Tropheini (belonging to the 'modern haplochromines' *sensu* Salzburger *et al.* 2005), and currently classified into six morphologically similar, albeit polyphyletic, species (Poll 1986; Sturmbauer *et al.* 2005). Sexual selection has been included in explanations of the rapid and sometimes convergent evolution of distinct colour patterns in the mostly allopatrically distributed variants (Yanagisawa & Nishida 1991; Sturmbauer & Meyer 1992; Salzburger *et al.* 2006), but apart from extreme parental investment by mouthbrooding females

* Author for correspondence (kristina.sefc@uni-graz.at).

The electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2006.3504> or via <http://www.journals.royalsoc.uk>.

(Schürch & Taborsky 2005), most *Tropheus* species lack some characteristics of sexually selected species, notably sexual dimorphism and social polygamy. Female *Tropheus* establish a pair bond with a chosen mate and draw resources from his territory for a period of up to three weeks before spawning. Feeding rate and several indices of physical condition, including the gonadosomatic index, are higher in paired than in solitary females, indicating that females cannot mature their ovaries in their own, smaller and possibly inferior, territories and depend on the nutritional resources of a male's large territory, where they forage actively under their mate's protection (Yanagisawa & Nishida 1991; Sturmbauer & Dallinger 1995). Sequences of courtship and spawning behaviour in *Tropheus* are similar to those displayed by other haplochromines (McElroy & Kornfield 1990), and include lead swimming by the male, quivering by both sexes, release and snapping up of eggs by the female, and nuzzling of the male's anal fin by the female (Nelissen 1976). Upon spawning, the female abandons the male territory and settles in an unoccupied site to mouthbrood. Females usually remain at the breeding site after release of the fry, and expand their territories as their feeding activity increases (Yanagisawa & Nishida 1991). Unlike in many polygynous haplochromines, *Tropheus* females are fully included in territorial and social interactions of the community, which also involves communication via sex-independent (colour) signals (Wickler 1969; Sturmbauer & Dallinger 1995).

The present study employs microsatellite markers for paternity analysis of *Tropheus moorii* broods, and determines whether the social pair bonds concur with genetic monogamy within breeding efforts, or whether extra-pair fertilization could increase the potential for sexual selection by enhancing the variance in male reproductive success (Jones *et al.* 2001). In several other Tropheini species, sneaking males have been observed to mingle with spawning pairs (Kuwamura 1987; Ochi 1993a,b), and we include a survey of paternity in several species of the tribe.

2. MATERIAL AND METHODS

(a) Sample collection and laboratory methods

Maternal families of *T. moorii* were collected from five locations along the southern shore of Lake Tanganyika, Zambia: Muzumwa ($n=5$; 08°41.97' S, 31°12.03' E), Tonga ($n=10$; 08°43.8' S, 31°8.4' E), Mbita Island ($n=2$; 08°45.06' S, 31°6.24' E), Chituta ($n=1$; 08°43.71' S, 31°9.35' E) and Kalambo ($n=1$; 08°36.51' S, 31°11.65' E). Embryos or fry and a fin clip of the mother were preserved in 99% ethanol, and total length of offspring was measured prior to DNA extraction. *Tropheus moorii* population samples from Muzumwa ($n=33$) and Wonzye ($n=23$) were used to evaluate marker polymorphism. Mouthbrooding mothers of seven additional species were also analysed: *Simochromis pleurospilus*, *Simochromis diagramma*, *Simochromis babaulti*, *Petrochromis fasciolatus* ($n=2$), *Petrochromis orthognatus*, *Gnathochromis pfefferi* and *Ctenochromis horei*.

DNA was extracted by proteinase K digestion, sodium chloride extraction and ethanol precipitation (Bruford *et al.* 1998) from fin clips and embryos after removal of the yolk sac. The families were genotyped at four microsatellite loci. TmoM11 (Zardoya *et al.* 1996) and UNH130 (Lee & Kocher 1996), as well as UME003 (Parker & Kornfield 1996) and Pzeb1 (Van Oppen *et al.* 1997), were simultaneously

amplified in multiplex PCR reactions containing 1 U DNA polymerase (BioTherm), 1 × reaction buffer (BioTherm) with 1.5 mM MgCl₂ in the reaction, 0.5 μM of each primer and 62.5 μM of each dNTP, under the following temperature regime: 2 min initial denaturation at 94 °C; 45 cycles of 30 s at 92 °C, 1 min at 50 °C, 1 min at 72 °C; 90 min final extension at 72 °C. PCR products and TAMRA 500 internal size standard (Applied Biosystems; ABI) were loaded on an ABI 377 automatic sequencer, and gels were analysed in GENESCAN v. 3.1.2. (ABI).

(b) Data analysis

Paternal alleles were inferred from the genotypes of the offspring and their mothers. In *T. moorii* (but not in the other members of the tribe Tropheini), locus Pzeb1 was problematic to score by allele size due to multiple stutter bands, addition of extra nucleotides (Magnuson *et al.* 1996) and allele size differences of one basepair; therefore, identity with the maternal alleles and the number of paternal alleles was determined for each *T. moorii* progeny array without sizing the peaks. An assessment of the efficacy of the markers to detect multiple paternity requires an estimate of allele frequencies in the populations from which the clutches were sampled. We calculated the exclusion probabilities (GERUD1.0; Jones 2001) and the likelihood to detect multiple paternity (GERUDSIM1.0; Jones 2001) for the clutches sampled from Muzumwa and Tonga, based on allele frequency data obtained from population samples from Muzumwa and Wonzye. The Wonzye population was used as reference for the Tonga families, as the two sampling locations are in close proximity, and no differentiation was detected between the Tonga sample consisting of maternal and inferred paternal genotypes and the Wonzye population ($F_{ST}=0.008$; $p>0.05$), whereas significant genetic differentiation exists between populations from Muzumwa and Wonzye ($F_{ST}=0.06$; $p<0.0001$). Population differentiation was estimated in ARLEQUIN (Schneider *et al.* 2000). Genotype frequencies in all locus-population combinations complied with Hardy-Weinberg expectations (GENEPOP v. 3.4; Raymond & Rousset 1995).

GERUDSIM creates simulated progeny arrays for a given number of progeny and fathers, based on the population allele frequencies, and then reconstructs the number of fathers contributing to the array from the simulated genotypes. The proportion of iterations, in which the reconstructed number of fathers is less than the actual number of fathers used to create the progeny array, indicates the probability to underestimate the number of fathers with the employed marker set. For each clutch size observed in the families from Muzumwa and Tonga, we estimated the likelihood to falsely infer single paternity when all but one offspring in the brood were sired by the same male from 10 000 iterated simulations. With only one offspring sired by an additional male, the simulations consider the most difficult scenario for the detection of multiple paternity, and provide a conservative evaluation of the marker efficacy, potentially underestimating the confidence in the analysis.

3. RESULTS

(a) Paternity analysis in *Tropheus moorii* broods

Clutch sizes of mouthbrooding *T. moorii* ranged from 5 to 13 offspring (mean 8.7, s.d. 2.52). Within each clutch, total length (L_T) varied by less than 0.1 cm, and broods

Table 1. Polymorphism and paternity exclusion probability of three microsatellite loci in the reference populations from Wonzye and Muzumwa.

locus	number of alleles		gene diversity		exclusion probability	
	Wonzye	Muzumwa	Wonzye	Muzumwa	Wonzye	Muzumwa
TmoM11	13	10	0.913	0.714	0.786	0.511
UNH130	16	14	0.937	0.904	0.824	0.764
UME003	16	12	0.926	0.875	0.810	0.730

ranged from 0.9 to 2.3 cm (mean 1.7 cm, s.d. 0.35 cm). No correlation was found between L_T and clutch size (Pearson's correlation coefficient $r = -0.09$), suggesting that mothers do not progressively lose offspring during the incubation period.

The three markers tested in the reference populations (TmoM11, UNH130 and UME003) were highly variable (table 1) and achieved high exclusion probabilities (cumulative P_E of 0.993 and 0.969 in Wonzye and Muzumwa, respectively). A fourth marker, Pzeb1, was not evaluated at population level, but scored in the maternal families (see §2). Therefore, allele frequency-based estimates of polymorphism were not calculated for Pzeb1, but high-observed heterozygosity (69.4% among the maternal and inferred paternal genotypes) indicates that the marker is informative for the present paternity study. In all but one of the surveyed families, the genotypes at these four loci were consistent with motherhood of the brooding female and a single, but different, sire for each clutch (see electronic supplementary material). In family T04-#7, genotypes at three loci were consistent with single paternity of the clutch, whereas at UME003, one offspring carried a third, non-maternal allele. Since at the other three loci the non-maternal alleles of this juvenile matched the paternal alleles inferred from the remaining brood, it is possible that the extra allele originated from a mutation rather than multiple paternity.

The probability of not detecting a second father when it sired only one offspring of the clutch ranged from 0.019 to 0.138 in the families from Tonga and Muzumwa (see electronic supplementary material). Accordingly, the probability to falsely infer single paternity for each of these clutches, when more than one male were involved in each case, is below 1.47×10^{-21} based on the three-locus genotypes. Note that the fourth marker applied to the families (Pzeb1) further increased the power of the analysis. We conclude that single paternity is prevalent in broods of *T. moorii*.

(b) Polyandry in the tribe *Tropheini*

A survey of the occurrence of polyandry in the tribe *Tropheini* was conducted in maternal families of seven additional species. The minimum number of fathers inferred from the four-locus genotypes of mothers and offspring was one in all broods except for one clutch of *P. fasciolatus*, in which up to four paternal alleles per locus contributed to the offspring genotypes (see electronic supplementary material). A second *P. fasciolatus* family, however, complied with single paternity of the brood. High levels of polymorphism among the observed maternal and inferred paternal genotypes (mean of three alleles per family and locus; percentage of heterozygous genotypes: TmoM11, 41.2%; UNH130, 88.2%;

UME003, 76.5%; Pzeb1, 64.7%) suggest that the markers are informative for paternity studies in these species.

4. DISCUSSION

(a) Monogamy, mating behaviour and parental investment

With one possible exception, the *T. moorii* broods surveyed in this study were sired by a single male each, implying that multiple paternity within broods occurs infrequently or not at all in this species. Among the other *Tropheini* surveyed in this study, only one brood of *P. fasciolatus* was sired by at least two males. Sneakers have been observed in related rock-dwelling *Tropheini* species such as *Pseudosimochromis curvifrons* and *S. diagramma* (Kuwamura 1987), *C. horei* (Ochi 1993a) and *G. pfefferi* (Ochi 1993b), but we know of no field observations of spawning in *P. fasciolatus*, and it remains unclear whether deliberate polyandry or sneaking underlie multiple paternity in this brood.

In contrast to the prevalence of single paternity in the studied *Tropheini*, multiple paternity is the rule rather than the exception in mouthbrooding cichlids of Lake Malawi (Kellogg *et al.* 1995, 1998; Parker & Kornfield 1996). Despite their close phylogenetic relatedness and otherwise similar life history, mating behaviour differs between *Tropheus* and the Lake Malawi haplochromines. Females of sand-dwelling cichlids in Lake Malawi have been observed to mate with multiple males for a single brood (McKaye 1991), and molecular analyses revealed multiple paternity in clutches of rock-dwelling species (*mbuna*), where spawning with different males had not been suspected from observational data (Parker & Kornfield 1996). Predation pressure and sneaky males have been discussed as causes of polyandry in Lake Malawi cichlids, especially with regard to sand-dwelling species, where disturbances by predators and sneakers reduce the length of individual mating encounters and encourage switches into other males' territories (Kellogg *et al.* 1995). By spawning in concealed breeding sites, the rock-dwelling *mbuna* of Lake Malawi are less vulnerable to intruders, and multiple paternity in some of these species has been ascribed to females actively seeking multiple mating partners as a bet-hedging strategy against imperfect mate choice (Kellogg *et al.* 1995; Parker & Kornfield 1996; Genner & Turner 2005). *Tropheus* always remain close to the substrate, browse epilithic algae, spawn openly on rock surfaces and dart into small caves and crevices for cover instantly when threatened. In contrast to the *mbuna* cichlids, the bonding that precedes spawning in *Tropheus* makes it more likely that a pair resumes spawning after a disturbance than that the female joins another male to complete her clutch. Furthermore, females are 'fickle' before pairing with one male, paying

repeated visits to several males' territories to assess the quality of the territory and/or male, and whereas no correlation between male body size and success of pairing was detected, removal experiments showed that previously solitary males acquired mates soon after they expanded their territories to incorporate parts of the now vacant neighbouring area (Yanagisawa & Nishida 1991). While the attachment to a particular male and his territory poses a constraint on polyandry, it is also conceivable that the prolonged courtship period and the attention to the value of a male's territory offer sufficient cues for a reliable assessment of male quality and thus render bet-hedging against suboptimal mates unnecessary.

Monogamy in fishes is generally correlated with bi-parental care of eggs and fry (Barlow 1986; Mock & Fujioka 1990; DeWoody *et al.* 2000; but see Reavis & Barlow 1998). Among cichlids, social monogamy is found in bi-parentally guarding substrate brooders, as well as in mouth brooding species in which males take over the wrigglers from females (Kuwamura 1986; Barlow 2000), but molecular proof of genetic monogamy has so far been delivered only for the Tanganyikan bi-parental mouth-brooder *Eretmodus cyanostictus* (Taylor *et al.* 2003). Maternal mouthbrooding entails highly skewed female investment in reproduction and is associated with polygamous mating systems, lekking and pronounced sexual dichromatism in other cichlid species (Kuwamura 1986). *Tropheus* males, however, invest in their mate's fertility by allowing females to harvest the nutritional resources essential for ovary development from their territory (Yanagisawa & Nishida 1991), and thus share in the costs of reproduction albeit without directly providing parental care.

(b) Sexual poly- and monochromatism and the role of colour signals in courtship, mating and social interactions

Male nuptial coloration of many sexually dichromatic haplochromines includes conspicuous yellow to orange spots surrounded by an outer ring on the anal fin. Wickler (1962) suggested that these markings stimulate the females to nuzzle the male's anal fin during spawning. When the female tries to grasp these 'egg-dummies', she inhales the sperm ejected by the male and ensures fertilization of the eggs within her mouth (Mrowka 1987). However, the function of egg spots is not yet fully understood (Barlow 2000), and there is evidence for alternative roles in attracting females and enhancing female fertility (Hert 1989) in some species. Elaborate egg mimics on the anal fin are considered a synapomorphy of the 'modern haplochromines' and their sister clade (*Astatoreochromis allaudi*), but have been lost secondarily in some deep-water lineages (Salzburger *et al.* 2005). Small spots lacking an outer ring on the anal fin are present in most variants of *Tropheus* (a 'modern haplochromine'), and probably represent the remnants of the—now lost—true egg spots present in the ancestors of *Tropheus* (Barlow 2000). Similarly, most haplochromines are sexually dimorphic, and dichromatism may have secondarily been lost in *Tropheus* (except for *Tropheus brichardi* and *Tropheus annectens*). Aside from its significance during courtship and mating, coloration in *Tropheus* has important communicative functions during social interactions, and both genders exhibit concurrent repertoires of

context-dependent colour patterns signalling motivation and social status (Wickler 1969; Nelissen 1976; Sturmbauer & Dallinger 1995). The social pressure involved in obtaining and maintaining territories (West-Eberhard 1983; Dominey 1984) seems to promote conspicuous dominant coloration in both males and females, and sexual dichromatism may not be affordable to females who must participate in the competition for territories for sustenance outside their courtship period (Kawanabe 1986; Yanagisawa & Nishida 1991).

(c) Excessive geographical colour variation in the absence of strong sexual selection

Although the degree of colour variation within *Tropheus* rivals that observed in the *mbuna* haplochromines of Lake Malawi, it was probably attained through a different mechanism. In Lakes Malawi and Victoria, closely related species differing solely in colour often coexist in sympatry (Genner *et al.* 1999; Seehausen & van Alphen 1999) and reproduce assortatively due to divergent female preferences (Van Oppen *et al.* 1998). It has been suggested that divergent or disruptive selection on male coloration could explain the diversity and geographical distribution of colour patterns within genera (e.g. Allender *et al.* 2003; but see Arnegard & Kondrashov 2004). In contrast, most of the *Tropheus* variants are distributed allopatrically, and where different morphs occur in the same location they belong to distinct mitochondrial lineages, suggesting secondary contact (Sturmbauer *et al.* 2005). Closely related, but allopatrically distributed, variants of *T. moorii* mate colour-assortatively both in the field following human-mediated admixis (Salzburger *et al.* 2006) and under experimental laboratory conditions (B. Egger 2005, unpublished data), but given the low potential for sexual selection in the species, it is unlikely that female preferences could trigger colour diversification in the absence of geographical isolation and give rise to sympatric 'sister morphs'. In the light of the above considerations, it is even questionable whether intersexual selection for male traits could have contributed significantly to the allopatric evolution of the manifold *Tropheus* variants.

5. CONCLUSIONS

By demonstrating genetic monogamy in *T. moorii*, the present study confirms behavioural observations of temporal pair bonding and social monogamy in *T. moorii*. The absence of extra-pair fertilizations that would introduce male reproductive variance into a socially monogamous system, the prevalence of territory size and/or quality over male morphology in determining female mate choice, the males' contribution to reproduction over and above the insemination of eggs, and the similar social roles of males and females possibly underlying sexual monochromatism in the taxon distinguish *T. moorii* from many other 'modern haplochromines', and make it unlikely that sexual selection played a similarly important role in the diversification of *Tropheus* as is hypothesized for the haplochromine radiation of Lakes Malawi and Victoria.

We thank the team at the Mpulungu Station of the Ministry of Agriculture, Food and Fisheries, Republic of Zambia, for their cooperation during fieldwork, and fishermen in

Mpulungu and at Kalambo Lodge for help with catching mouthbrooding females. We are grateful to the reviewers for comments and suggestions on a previous version of this manuscript. Thanks to Nina Duftner, Ismene Fertschai and Stephan Koblmüller for help and companionship in the field. The work was supported by the Austrian Science Foundation (grant P17380-B06).

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.
- Avise, J. C., Jones, A. G., Walker, D. & DeWoody, J. A. 2002 Genetic mating systems and reproductive natural histories of fishes: lessons for ecology and evolution. *Annu. Rev. Genet.* **36**, 19–45. (doi:10.1146/annurev.genet.36.030602.090831)
- Barlow, G. W. 1986 Mate choice in the monogamous and polychromatic Midas cichlid, *Cichlasoma citrinellum*. *J. Fish Biol.* **29A**, 123–133. (doi:10.1111/j.1095-8649.1986.tb05004.x)
- Barlow, G. W. 1991 Mating systems among cichlid fishes. In *Cichlid fishes. Behaviour, ecology and evolution* (ed. M. H. A. Keenleyside), pp. 173–190. New York, NY: Chapman & Hall.
- Barlow, G. W. 2000 The cichlid fishes: nature's grand experiment in evolution. Cambridge, MA: Perseus.
- Bruford, M. W., Hanotte, O., Brookfield, J. F. Y. & Burke, T. 1998 Multilocus and single-locus DNA fingerprinting. In *Molecular genetic analysis of populations: a practical approach* (ed. A. R. Hoelzel), pp. 287–336. Oxford, UK: IRL Press.
- Coyne, J. A. & Orr, H. A. 2004 *Speciation*. Sunderland, MA: Sinauer Associates.
- Danley, P. & Kocher, T. 2001 Speciation in rapidly diverging systems: lessons from Lake Malawi. *Mol. Ecol.* **10**, 1075–1086. (doi:10.1046/j.1365-294X.2001.01283.x)
- DeWoody, J. A., Fletcher, D. E., Wilkins, S. D., Nelson, W. S. & Avise, J. C. 2000 Genetic monogamy and biparental care in an externally fertilizing fish, the largemouth bass (*Micropterus salmoides*). *Proc. R. Soc. B* **267**, 2431–2437. (doi:10.1098/rspb.2000.1302)
- Dierkes, P., Taborsky, B. & Kohler, U. 1999 Reproductive parasitism of broodcare helpers in a cooperatively breeding fish. *Behav. Ecol.* **10**, 510–515. (doi:10.1093/beheco/10.5.510)
- 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. 231–249. Orono, ME: University of Maine at Orono Press.
- Fryer, G. & Iles, T. D. 1972 *The cichlid fishes of the great lakes of Africa. Their biology and evolution*. Edinburgh, UK: Oliver and Boyd.
- Genner, M. J. & Turner, G. F. 2005 The mbuna cichlids of Lake Malawi: a model for rapid speciation and adaptive radiation. *Fish Fish.* **6**, 1–34.
- Genner, M. J., Turner, G. F., Barker, S. & Hawkins, S. J. 1999 Niche segregation among Lake Malawi cichlid fishes? Evidence from stable isotope signatures. *Ecol. Lett.* **2**, 185–190. (doi:10.1046/j.1461-0248.1999.00068.x)
- Griffiths, S. C., Owens, I. P. F. & Thuman, K. A. 2002 Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Mol. Ecol.* **11**, 2195–2212. (doi:10.1046/j.1365-294X.2002.01613.x)
- Hert, E. 1989 The function of egg-spots in an African mouthbrooding cichlid fish. *Anim. Behav.* **37**, 726–732. (doi:10.1016/0003-3472(89)90058-4)
- Hughes, C. 1998 Integrating molecular techniques with field methods in studies of social behavior: a revolution results. *Ecology* **79**, 383–399.
- Jones, A. G. 2001 Gerud 1.0: a computer program for the reconstruction of parental genotypes from progeny arrays using multilocus DNA data. *Mol. Ecol. Notes* **1**, 215–218. (doi:10.1046/j.1471-8278.2001.00062.x)
- Jones, A. G., Walker, D., Kvarnemo, C., Lindström, K. & Avise, J. C. 2001 How cuckoldry can decrease the opportunity for sexual selection: data and theory from a genetic parentage analysis of the sand goby, *Pomatoschistus minutus*. *Proc. Natl Acad. Sci. USA* **98**, 9151–9156. (doi:10.1073/pnas.171310198)
- Katoh, R., Munehara, H. & Kohda, M. 2005 Alternative male mating tactics of the substrate brooding cichlid *Telmatochromis temporalis* in Lake Tanganyika. *Zool. Sci.* **22**, 555–561. (doi:10.2108/zsj.22.555)
- Kawanabe, H. 1986 Territorial behaviour of *Tropheus moorei* (Osteichthyes: Cichlidae). *Afr. Stud. Monogr.* **1**, 101–108.
- Kellogg, K. A., Markert, J. A., Stauffer Jr, J. R. & Kocher, T. D. 1995 Microsatellite variation demonstrates multiple paternity in lekking cichlid fishes from Lake Malawi, Africa. *Proc. R. Soc. B* **260**, 79–84.
- Kellogg, K. A., Markert, J. A., Stauffer Jr, J. R. & Kocher, T. D. 1998 Intraspecific brood mixing and reduced polyandry in a maternal mouth-brooding cichlid. *Behav. Ecol.* **9**, 309–312.
- 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)
- Kondrashov, A. S. & Shpak, M. 1998 On the origin of species by means of assortative mating. *Proc. R. Soc. B* **265**, 2273–2278. (doi:10.1098/rspb.1998.0570)
- Konings, A. 1998 *Tanganyikan cichlids in their natural habitat*, 2nd edn. El Paso, TX: Cichlid Press.
- Kuwamura, T. 1986 Parental care and mating systems of cichlid fishes in Lake Tanganyika: a preliminary field survey. *J. Ethol.* **4**, 129–146.
- Kuwamura, T. 1987 Male mating territory and sneaking in a maternal mouthbrooder, *Pseudosimochromis curvifrons* (Pisces; Cichlidae). *J. Ethol.* **5**, 203–206.
- Kuwamura, T. 1997 The evolution of parental care and mating systems among Tanganyikan cichlids. In *Fish communities in Lake Tanganyika* (ed. H. Kawanabe, M. Hori & N. Makoto), pp. 57–86. Kyoto, Japan: Kyoto University Press.
- Lee, W.-J. & Kocher, T. D. 1996 Microsatellite DNA markers for genetic mapping in *Oreochromis niloticus*. *J. Fish Biol.* **49**, 169–171.
- Maan, M. M., Seehausen, O., Söderberg, L., Johnson, L., Ripmeester, E. A. P., Mrosso, H. D. J., Taylor, M. I., van Dooren, T. J. M. & van Alphen, J. J. M. 2004 Intraspecific sexual selection on a speciation trait, male coloration, in the Lake Victoria cichlid *Pundamilia nyererei*. *Proc. R. Soc. B* **271**, 2445–2452. (doi:10.1098/rspb.2004.2911)
- Magnuson, V. L., Ally, D. S., Nylund, S. J., Karanjawala, Z. E., Rayman, J. B., Knapp, J. I., Lowe, A. L., Ghosh, S. & Collins, F. S. 1996 Substrate nucleotide-determined non-templated addition of adenine by Taq DNA polymerase: implications for PCR-based genotyping and cloning. *BioTechniques* **21**, 700–709.

- McElroy, D. M. & Kornfield, I. 1990 Sexual selection, reproductive behavior, and speciation in the mbuna species flock of Lake Malawi (Pisces: Cichlidae). *Environ. Biol. Fish.* **28**, 273–284. (doi:10.1007/BF00751041)
- 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. New York, NY: Chapman & Hall.
- Mock, D. W. & Fujioka, M. 1990 Monogamy and long-term pair bonding in vertebrates. *Trends Ecol. Evol.* **5**, 39–43. (doi:10.1016/0169-5347(90)90045-F)
- Mrowka, W. 1987 Filial cannibalism and reproductive success in the maternal mouthbrooding cichlid fish *Pseudocrenilabrus multicolor*. *Behav. Ecol. Sociobiol.* **21**, 257–265. (doi:10.1007/BF00292507)
- Nelissen, M. 1976 Contribution to the ethology of *Tropheus moorii* Boulenger (Pisces, Cichlidae) and a discussion of the significance of its colour pattern. *Rev. Zool. Afr.* **90**, 17–29.
- Ochi, H. 1993a Mate monopolization by a dominant male in a multi-male social group of a mouthbrooding cichlid, *Ctenochromis horei*. *Jpn. J. Ichthyol.* **40**, 209–218.
- Ochi, H. 1993b Maintenance of separate territories for mating and feeding by males of a maternal mouthbrooding cichlid, *Gnathochromis pfefferi*, in Lake Tanganyika. *Jpn. J. Ichthyol.* **40**, 173–182.
- Panhuis, T. M., Butlin, R., Zuk, M. & Tregenza, T. 2001 Sexual selection and speciation. *Trends Ecol. Evol.* **16**, 364–371. (doi:10.1016/S0169-5347(01)02160-7)
- Parker, A. & Kornfield, I. 1996 Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Environ. Biol. Fish.* **47**, 345–352. (doi:10.1007/BF00005049)
- Pauers, M. J., McKinnon, J. S. & Ehlinger, T. J. 2004 Directional sexual selection on chroma and within-pattern colour contrast in *Labeotropheus fuelleborni*. *Proc. R. Soc. B* **271**(Suppl. 6), 444–447. (doi:10.1098/rsbl.2004.0215)
- Poll, M. 1986 Classification des cichlidae du lac Tanganika. Tribus, genres et espèces. *Acad. R. Belg. Mem. Cl. Sci.* **45**, 1–163.
- Raymond, M. & Rousset, F. 1995 GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.* **86**, 248–249.
- Reavis, R. H. & Barlow, G. W. 1998 Why is the coral-reef fish *Valencia strigata* (Gobiidae) monogamous? *Behav. Ecol. Sociobiol.* **43**, 229–237. (doi:10.1007/s002650050487)
- 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)
- Salzburger, W., Niederstätter, H., Brandstätter, A., Berger, B., Parson, W., Snoeks, J. & Sturmbauer, C. 2006 Colour-assortative mating among populations of *Tropheus moorii*, a cichlid fish from Lake Tanganyika, East Africa. *Proc. R. Soc. B* **273**, 257–266. (doi:10.1098/rspb.2005.3321)
- Schneider, S., Roessli, D. & Excoffier, L. 2000 Arlequin ver. 2.000: a software for population genetics data analysis. Geneva, Switzerland: Genetics and Biometry Laboratory, University of Geneva.
- Schupke, P. 2003 *African cichlids II: Tanganyika I: Tropheus*. Germany: Aqualog, A.C. S GmbH.
- Schürch, R. & Taborsky, B. 2005 The functional significance of buccal feeding in the mouthbrooding cichlid *Tropheus moorii*. *Behaviour* **142**, 265–281.
- Seehausen, O. & van Alphen, J. J. M. 1999 Can sympatric speciation by disruptive sexual selection explain rapid evolution of cichlid diversity in Lake Victoria? *Ecol. Lett.* **2**, 262–271. (doi:10.1046/j.1461-0248.1999.00082.x)
- Seehausen, O., Witte, F., van Alphen, J. J. M. & Bouton, N. 1998 Direct mate choice maintains diversity among sympatric cichlids in Lake Victoria. *J. Fish Biol.* **53**, 37–55.
- Sturmbauer, C. 1998 Explosive speciation in cichlid fishes of the African Great Lakes: a dynamic model of adaptive radiation. *J. Fish Biol.* **53**, 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., Koblmüller, 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)
- Sugg, D. W. & Chesson, R. K. 1994 Effective population sizes with multiple paternity. *Genetics* **137**, 1147–1155.
- Taylor, M. I., Morley, J. I., Rico, C. & Balshine, S. 2003 Evidence of genetic monogamy and female-biased dispersal in the parental mouthbrooding cichlid *Eretmodus cyanostictus* from Lake Tanganyika. *Mol. Ecol.* **12**, 3173–3177. (doi:10.1046/j.1365-294X.2003.01964.x)
- Van Oppen, M. J. H., 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. H., 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)
- West-Eberhard, M. J. 1983 Sexual selection, social competition, and speciation. *Q. Rev. Biol.* **58**, 155–183. (doi:10.1086/413215)
- Wickler, W. 1962 ‘Egg-dummies’ as natural releasers in mouth-brooding cichlids. *Nature* **194**, 1092–1093.
- Wickler, W. 1969 Zur Soziologie des Brabantbuntbarsches, *Tropheus moorei* (Pisces, Cichlidae). *Z. Tierpsychol.* **26**, 967–987.
- Yanagisawa, Y. & Nishida, M. 1991 The social and mating system of the maternal mouthbrooder *Tropheus moorii* (Cichlidae) in Lake Tanganyika. *Jpn. J. Ichthyol.* **38**, 271–282.
- 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.