

Individual variation in male mating preferences for female coloration in a polymorphic cichlid fish

Michele E.R. Pierotti,^{a,b,c} Mairi E. Knight,^d Simone Immler,^e Nicola J. Barson,^f George F. Turner,^a and Ole Seehausen^{b,c}

^aMolecular Ecology and Evolution Group, Department of Biological Sciences, University of Hull, Cottingham Road, Hull HU6 7RX, UK, ^bAquatic Ecology and Macroevolution, Institute of Zoology, University of Bern, Baltzerstrasse 6, CH-3012 Bern, Switzerland, ^cEAWAG Center for Ecology, Evolution and Biogeochemistry, 79 Seestrasse, CH-6047 Kastanienbaum, Switzerland, ^dTerrestrial Ecology Research Group, School of Biological Sciences, University of Plymouth, Plymouth PL4 8AA, UK, ^eEvolution & Behaviour Research Group, Department of Animal and Plant Sciences, University of Sheffield, Western Bank S10 2TN, Sheffield, UK, and ^fCentre for Ecological and Evolutionary Synthesis, Department of Biology, University of Oslo, PO Box 1066 Blindern, N-0316 Oslo, Norway

Female color polymorphisms are common in the cichlid species radiations of Lake Victoria and Lake Malawi. According to theory, when a population harbors variation in sex-determining factors, polymorphism in female-linked coloration might generate individual variation in male mating preferences for female color morphs. We tested whether individual males exhibit consistent mating preferences for female color morphs in the Lake Malawi cichlid *Pseudotropheus (Maylandia) 'zebra gold'*, a species polymorphic for female coloration and sex determination. We also explored whether male mating preferences could be predicted by maternal coloration or were acquired by imprinting on siblings' coloration. We found large individual variation in the strength and direction of male preferences for sex-linked female color patterns. Male mating preferences could be predicted by the mother's color morph and were not affected by visual imprinting. These findings represent the first evidence of male choice on sex-linked female coloration in a Lake Malawi cichlid. Our analysis indicates a strong genetic component to male preference for female coloration and large individual variation in the strength and direction of male mating preferences. Within-population variation in innate mating preferences might have important implications in cichlid fish species radiations. *Key words:* cichlid, male preferences, mate choice, sex determination, sex ratio. [*Behav Ecol* 19:483–488 (2008)]

The study of within-population variation in sexually selected traits has been a long tradition dating back to Darwin (1871; Andersson 1994). It is then somewhat surprising that within-population variation in mate preferences has not followed a similar fate. Individual variation is a prerequisite for selection to act on preferences and can have profound consequences on the intensity and direction of sexual selection (Jennions and Petrie 1997; Wagner 1998). Moreover, within-population variation in mate preferences has important bearing on speciation studies (e.g., Turner and Burrows 1995; Higashi et al. 1999; Takimoto et al. 2000; Arnegard and Kondrashov 2004). Whereas there is some empirical evidence for divergent female mating preferences within natural populations (Sappington and Taylor 1990; Greene et al. 2000; Morris et al. 2003), male mating preferences have been only recently the subject of empirical and theoretical work (e.g., Bonduriansky 2001; Servodio and Lande 2006).

Seehausen et al. (1999) found evidence of divergent male mating preferences within a population of the Lake Victoria cichlid fish *Neochromis omnicaeruleus*, in which 3 color morphs can co-occur at various frequencies in different populations.

One color morph (plain [P] morph) shows the typical pattern of dark vertical bars common in many other rock-dwelling cichlid species of Lakes Victoria and Malawi. In the other 2 morphs, vertical bars are substituted by black blotches on an orange background (orange-blotched [OB] morph) or on a yellow–white background (piebald [WB] morph). The authors showed that both blotched morphs are carriers of different dominant female determiners (sex reversal factors) linked to the blotch genes. As a consequence, matings between P and blotched (WB/OB) individuals can result in heavily female-biased broods. Mate choice trials in the lab revealed male and female mating preferences for the different color morphs, and morph frequency distribution in the wild was consistent with nonrandom mating between color morphs. Seehausen et al. (1999) concluded that *N. omnicaeruleus* might represent a case of arrested incipient speciation by selection on sex reversal and sexual selection.

Male mating preferences, as found in *N. omnicaeruleus*, are not expected to be common among the haplochromine cichlid fishes of Lakes Victoria and Malawi. The haplochromine cichlids of the East African lakes are polygynandrous female mouthbrooders: whereas male investment is limited to courtship and gamete production, females incur high costs of parental care during the mouthbrooding period, when they refrain from feeding and are possibly more exposed to predators. Given such highly asymmetric investment, strong female choice is expected but the reverse seems unlikely. Male choice

Address correspondence to M.E.R. Pierotti. E-mail: michele.pierotti@eawag.ch.

Received 24 October 2006; revised 10 December 2007; accepted 11 December 2007.

for female ornamentation in non sex–role–reversed fish species has been rarely described (Amundsen and Forsgren 2001, 2003; Basolo and Delaney 2001; Craig and Foote 2001), but this might also be partly due to a research bias (Houde 2001).

To date, there was no evidence that female sex-linked color polymorphisms are the target of mate choice in other cichlid fish radiations, that is, Lake Malawi cichlids, or in fact that individual variation exists in male mating preferences for alternative female color morphs in any East African cichlid radiation.

The mechanism of mate preference inheritance can influence the evolutionary trajectory of a sexually selected trait and possibly the likelihood and speed of species divergence by sexual selection (Laland 1994; Grant PR and Grant BR 1997; Price 1998; Lachlan and Servadio 2004; Albert 2005; Verzijden et al. 2005). Cichlid fishes have been the object of intense research in sexual selection, and while evidence for genetic inheritance of mate preferences has only recently started to emerge (Haesler and Seehausen 2005), the existence of sexual imprinting in cichlids has long been invoked. Earlier studies reported an influence of parental color morph on female mate choice in the neotropical cichlid *Archocentrus nigrofasciatus* (Weber PG and Weber SP 1976; Siepen and Crapon de Caprona 1986). Siblings' coloration seems also to be implicated in the development of female mating preferences in *Hemichromis bimaculatus* (Kop and Heuts 1973), *Astatotilapia burtoni* (Sjölander and Fernö 1973), and *A. nigrofasciatus* (Weber PG and Weber SP 1976). In the Lake Malawi *mbuna* cichlids, there is no parent–offspring interaction after the offspring leave the mother's mouth. Once released, mother and fry dissolve their bond and fry rush to the nearest natural shelter to avoid predation (Genner and Turner 2005). Therefore, there is very little opportunity for fry to imprint on mother's color pattern. However, imprinting on sibs' coloration might take place in this early stage of free swimming.

We studied male mating preferences in the blotch polymorphic *Pseudotropheus (Maylandia)* 'zebra gold', a species belonging to the Lake Malawi cichlid fish radiation. Previous work has established within-population variation in sex-determining factors and the association between dominant female sex determiners with dominant blotch coloration in this and related species (Holzberg 1978; Knight 1999). Here, we tested whether male mating preferences for female color morphs have evolved in this species and whether there is individual variation in strength and direction of mating preferences within a population. We also examined the mechanisms of preference acquisition by testing whether male mating preferences have a genetic basis or are acquired by imprinting on sibs' coloration.

MATERIALS AND METHODS

Study species

Pseudotropheus (Maylandia) 'zebra gold' (also known by the junior synonym *Metriaclima*; e.g., Stauffer et al. 1997) is an undescribed species of rock-dwelling ephilitic algae scrapers. Two color morphs occur in the population (Nkhata Bay) considered in this study: a "gold" morph (P), with males gold-yellow with 6–7 dark vertical bars and females brown with dark vertical bars, and an "orange-blotched" (OB) morph with females orange with irregular black blotches; OB males are extremely rare (Konings 2001). Female OB blotched coloration is dominant in *P.* 'zebra gold' and in other haplochromine species (Holzberg 1978; Knight 1999; Seehausen et al. 1999). In *Pseudotropheus (Maylandia) zebra*, Streebman et al. (2003) recently identified a single genomic region responsible for OB coloration, in physical linkage with the cichlid proto-oncogene *c-ski1* (Huang et al. 1999).

Housing conditions

Experimental males were kept in individual 19 × 19 × 18 cm aquaria allowing no visual contact of the test males with either female color morph. Females were kept in a female population tank. All aquaria were maintained at 26 ± 2 °C and illuminated with daylight fluorescent tubes on a 12:12 h light:dark cycle. Fish were fed twice a day with flake food and a vitamin-enriched mix of mashed prawns and peas. Males and females were tagged with a passive identification transponder (PIT) for individual identification.

A pilot study was conducted in 2001 on 14 individual males to assess whether males sired by P or OB mothers differ in their mating preferences for either female color morph. Seven males sired by wild-caught P parents (from a large laboratory-maintained P stock) and 7 males sired by wild-caught P father and OB mother (from a large laboratory-maintained OB stock) were tested 1–3 times each with a set of 4 P and 4 OB females. Male courtship behavior (see below for details) toward females of each morph was recorded for 15 min. During these trials, males and females could freely interact. For this reason, male mating preferences by visual cues could not be disentangled from effects of chemical and possibly acoustic communication.

A restricted maximum likelihood (REML) linear mixed model, which allows the treatment of unbalanced designs, was performed on the arcsine-transformed proportions of darts. Female morph and male pedigree were considered as fixed factors and female group and male identity as random factors. The analysis suggested a significant interaction between male pedigree and female morph (REML: Wald $F = 31.67$, $P < 0.001$). A standardized mean preference score was calculated as the ratio (dart displays to P female – dart displays to OB female)/total number of dart displays. A Mann–Whitney test on individual mean display scores showed that males sired by a P mother courted more P females than males sired by an OB mother ($U = 8.5$, $P = 0.038$).

This preliminary test had limited heuristic scope as it could not control for the influence of nonvisual communication on male choice for females of different color morph. The results might have been due to nonvisual male preferences (chemical, acoustic, etc.) or by the interaction of visual and nonvisual cues. Moreover, the design did not allow the assessment of individual male mate preferences. Therefore, the preliminary observations prompted us to design an experiment aiming at isolating visual male preferences as well as identifying variation in individual male mating preferences for female coloration. We controlled for potential imprinting on mother's coloration by retrieving fry from their mother's mouth before natural release (see below for details) and imprinting on mother's nonvisual cues by testing male offspring mating preferences in visual-only designs. Finally, we tested the effects of imprinting on sibs' coloration with a split-brood cross-sib fostering design.

Mode of inheritance of male preference

Experimental males were the sons of P morph fathers and either P morph mothers or OB morph mothers. P morph parents derived from an experimental P line and OB morph mothers from an experimental OB line were unrelated to individuals used in the pilot study.

To prevent any visual contact between mother and offspring, fry were gently removed from their mother's mouth after a week of incubation, that is, well before they would be naturally released by the mother (18–24 days). A split-brood cross-sib fostering design was used. Both P × P and OB × P clutches were split in 2 rearing treatments, resulting in 4 experimental

groups: individuals were exposed to either P sibs only or to sibs of both morphs. Families from P parents ($P \times P$) contain only individuals with P color patterns; families from heteromorphic pairs ($OB \times P$) are comprised of both “P” and “OB” phenotypes. Fry of P and OB morphs are initially nonblotched and indistinguishable. OB morphs begin developing the first melanin spots only after about 11 weeks post fertilization. For this reason, OB-only rearing treatments cannot be made. Offspring were removed from “P sibs-only” treatments once they showed the first signs of melanin spot development. Blotched fry at the same early stage of color pattern development was added to experimental clutches from $P \times P$ parents and assigned to OB + P rearing treatment.

Male choice trials

Two 14-L aquaria were placed at the short ends of the 2720-L experimental tank. This was fitted with a heater, kept at 26 °C, an external filter, and a halved terracotta flowerpot placed in the center as shelter and spawning site.

Males were introduced into the experimental aquarium and left to acclimatize overnight. The following day, a brown barred female (morph P) and an OB female were introduced each in one of the 2 small aquaria. These were then covered with a lid to exclude water or spray being exchanged between compartments. Therefore, no chemical communication was allowed between experimental males and females during the trials. Any chemical signal potentially exchanged by a mother and its fry during early mouthbrooding is unlikely to influence males' visual preferences unless genetic variation for pleiotropic effects or linkage between maternal chemical signals and male visual preferences was present.

For each trial, a different set of females was used and every male was only tested once with each female. Females were size matched (within 5% standard length difference) and the 2 morphs randomly allocated to the small aquaria.

Male behavior was scored for 15 min from its first encounter with a female, defined as approaching a female to a distance below 20 cm. Trials in which the males did not encounter both females and trials in which males did not court were discarded from the analyses. Male behavioral patterns recorded were neutral encounter, courtship displays (lateral display, dart, quiver, and lead swim), and aggressive displays (frontal display and butting) (Baerends and Baerends-van Roon 1950). For the analyses, we chose the behavioral elements that can be always unequivocally attributed to courtship (darts) and aggression (butting) (Pierotti MER, personal observation).

Gravidity stage was assessed by swelling of the female abdomen using the 5-point scale method of Seehausen and van Alphen (1998). Only ripening or fully ripe females (stages 3–5) were used in the experiments. After a trial, standard length (to the nearest 0.5 mm) and weight (to the nearest 0.1 g) were measured for each female.

Statistical analysis

Each experimental male was tested with 6–8 different female pairs, and a weighted analysis of variance with logit-transformed proportion data (number of displays toward P females/total number of displays) was used to assess variation of preference between males.

In addition, we calculated repeatability for male mating preference (Lessells and Boag 1987). To examine whether male mating preference (inferred from dart counts) could be predicted by mothers' genotype or by sibs' coloration, we fitted a generalized mixed-effect model (GLMM) with a binomial distribution (Sokal and Rohlf 1995) and logit link function based on Laplace approximation (lme4 package in R; Bates and Sarkar 2006). Male identity was included as random effect to account for repeated measurements (Hedeker 2005). The significance of each term in the models was assessed using Wald statistics (Sokal and Rohlf 1995). We obtained the minimal model by exclusion of the nonsignificant 2-way interactions between terms. To assess the strength of the effects, we calculated effect size d (Cohen 1988; Nakagawa 2004) and the respective confidence limits (Smithson 2003), where confidence intervals (CIs) including d indicate statistical significance.

Individual variation in male aggression behavior toward either female morph was tested as described for male preference. To identify a potential aggression bias toward a female color morph, we performed a GLMM model including male identity as a random factor and tested the overall intercept against zero.

RESULTS

Eighty-four preference choice tests on 12 experimental males were performed. Males courted 1 female morph, only, in 47.6% of the trials (see also Table 1). We found large individual variation in male mating preferences ($F = 5.80$, $P < 0.0001$, mean squares among males = 198, mean squares within males = 34; Figure 1). Repeatability for individual male mating preferences was $r = 0.39$.

Table 1

Treatment design and male IDs for the test of male preference mode of inheritance, number of trials per individual male, mean number of courtship displays (darts) per trial per individual (mean \pm standard error [SE]), and proportion of trials in which only one of the 2 female morphs was courted

Pedigree ($\text{♀ morph} \times \text{♂}$)	Rearing environment	Male	Trials n	Darts per trial (mean \pm SE)	Single female courted
$P \times P$	P	AA1	6	12.0 \pm 2.13	0.83
		AA2	8	4.7 \pm 1.47	0.86
		AA3	7	17.3 \pm 5.47	0.29
	P + OB	AB1	6	13.7 \pm 3.05	0.50
		AB2	8	7.0 \pm 2.22	0.50
$OB \times P$	P	BA1	8	14.2 \pm 1.77	0.50
		BA2	8	15.2 \pm 2.73	0.63
		BA3	6	3.2 \pm 0.95	0.83
	P + OB	BB1	7	18.4 \pm 1.70	0.00
		BB2	6	8.9 \pm 2.23	0.14
		BB3	7	17.1 \pm 2.28	0.00
		BB4	7	5.2 \pm 3.01	0.80

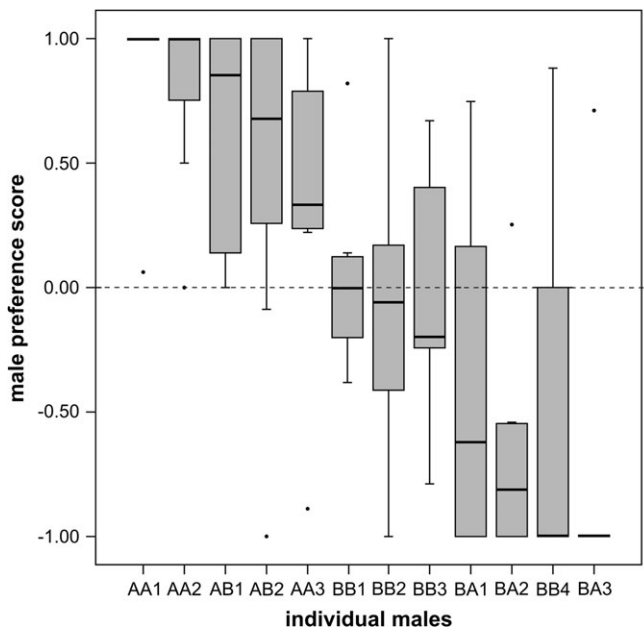


Figure 1

Individual male mating preference scores (dart displays to P female – dart displays to OB female/total number of dart displays). Positive preference score values indicate male preference for P females, and negative values indicate male preference for OB females. Three-digit codes indicate male treatment group and identity, as given in Table 1. Data are given as box plot diagrams showing medians (middle line in the boxes), first and third quartiles (boxes) and range (whiskers). Outliers (filled circles) are also shown.

The GLMM revealed that imprinting on sibs' coloration had no significant effect on male preference ($z = 1.42$, $P = 0.16$) nor was there any significant interaction between imprinting and male pedigree, that is, mother's color morph ($z = 1.78$, $P = 0.08$). Male preferences were determined by the males' pedigree (minimum model including only male pedigree: $z = -2.96$, $P = 0.003$; effect size $d = 0.66$; CI: 0.21–1.11) (Figure 1).

Males displayed aggressive behavior in 39 trials and 2 males did not exhibit any aggressive behavior toward females (males BA2 and BB1, Table 1 and Figure 1). The overall intercept of the GLMM model was significantly different from zero ($z = 3.26$, $P = 0.001$) indicating an aggression bias toward P morph females. There was no significant effect of a male's pedigree on its aggressive behavior toward either female morph when using the minimal adequate model ($z = 1.47$, $P = 0.14$). The effect of imprinting on aggression bias was significant in the model including pedigree ($z = 2.42$, $P = 0.01$). This effect of imprinting was nonsignificant when the pedigree term was removed ($z = -1.72$, $P = 0.09$), and there was no significant interaction between imprinting and pedigree ($z = 1.70$, $P = 0.09$).

DISCUSSION

Female mate choice on male nuptial coloration is frequently cited as an important diversifying force in the extraordinary radiations of East African cichlids (e.g., Seehausen et al. 1997; Jordan et al. 2003; Pauers et al. 2004). However, so far little attention has been paid to the role male mate choice might play although theoretical modeling has shown that under certain conditions male mating preferences could generate evolutionary dynamics leading to rapid speciation in sympatry (Lande et al. 2001; Kocher 2004).

Our results provide the first evidence of male mating preferences for female coloration in a polymorphic cichlid species

from Lake Malawi. We show that the coexistence of mating preferences for alternative values of a trait in the other sex in a single population is not an unrealistic condition of models of sympatric speciation (Arnegard and Kondrashov 2004). It might not be coincidental that such variation in the direction and strength of consistent repeatable mating preferences for different female morphs occurs in a member of a rapidly speciating cichlid lineage (Greenwood 1974; Kocher 2004; Genner and Turner 2005).

Males from plain mothers (P) preferred to court plain females, males from blotched mothers (OB) exhibited either no preference or a preference for blotched females, and male preference variation was best predicted by mother's color morph, suggesting genetic inheritance of the preference with no apparent influence of rearing experience on male mating preference development. The individual variation in male preferences for female coloration measured in our experiment cannot be attributed to any maternal effect in the form of imprinting on mother's coloration because we retrieved experimental males from their mother's mouth before natural release, excluding any possible visual contact between mother and offspring. Moreover, our design, precluding any nonvisual communication between males and stimulus females during mating preference trials, controls for any maternal influence in the form of imprinting on morph-specific nonvisual (e.g., olfactory) cues that might have taken place during the mouth-brooding period.

That male preferences for female color morphs have evolved in the context of sexual selection is also suggested by the results of Knight and Turner (1999) on the species *P. (Maylandia) 'zebra gold'* (our study species), *P. (Maylandia) zebra*, and *Pseudotropheus (Maylandia) callainos*, all 3 comprising blotched female morphs and living sympatrically at Nkhata Bay, Lake Malawi. The authors explored the possibility that male species recognition might contribute to the observed complete reproductive isolation between these closely related sympatric taxa. Knight and Turner (1999) found that *P. zebra* and *P. 'zebra gold'* males were unable to distinguish conspecific from heterospecific females of either color morph and courted both. Although individual variation in preferences was not considered in that study, these results indicate that male mating preferences in all 3 female polymorphic species play little role in species recognition.

The same female visual stimulus triggering male mate preference variation had a very different effect on male aggressive behavior. Male aggression preferences for female color morphs appeared to be decoupled from male mating preferences. Males exhibited a general aggression bias toward the P morph, attacking P females more than OB females. This general trend might perhaps originate from negative frequency-dependent selection mediating aggressive behavior toward different color morphs. OB females are less common than P females in all *P. 'zebra gold'* populations, and OB males are extremely rare (Konings 2001). In the context of resource defense under predation risk, it might be adaptive to have a search image for the invading rival of the most common morph. This might also give a blotched (OB) morph a selective advantage in the early stages of invasion of a P population because individuals with the novel color patterns (blotch) would initially face less aggression from the predominant P morph (Mikami et al. 2004; Seehausen and Schluter 2004). However, our experiment was not designed to examine differential aggression toward color morphs. Within-sex aggression experiments are needed to assess whether this pattern of aggression bias evolved in contexts other than sexual selection (e.g., ecological competition) or is a secondary effect of intra-sexual selection, in which males are selected to defend a spawning territory attacking males of the more common morph (P).

Finally, the female color and male preference polymorphism of *P. (Maylandia) 'zebra gold'* shows striking similarities with the *N. omnicaeruleus* polymorphism studied by Seehausen et al. (1999). In both systems, variation in male mating preferences for either plain or blotched females is present. In *N. omnicaeruleus*, Seehausen et al. (1999) proposed that male mating preferences might have evolved by sex ratio selection to avoid matings with females carrying dominant female determiners in linkage with blotched coloration. Knight (1999) found evidence of dominant female determiners associated with blotch in our study species, *P. 'zebra gold'*, and in its sister species *P. zebra*. Hence, similar forces might have shaped the evolution of male mating preferences in both the Lake Victoria cichlid *N. omnicaeruleus* and the Lake Malawi cichlid *P. (Maylandia) 'zebra gold'*.

Autosomal factors overriding the effects of dominant female-determining genes have been found both in *N. omnicaeruleus* (Seehausen et al. 1999) and in *P. 'zebra gold'* (Knight 1999). Males carrying such male-rescue factors are not affected by the sex ratio distortion deriving from mating with blotched females (i.e., carriers of dominant female determiners). Male mating preference variation might then be coupled to the possession or lack of male-rescue factors. There is preliminary evidence that blotched females are socially dominant over plain ones in *N. omnicaeruleus* and that this dominance is a pleiotropic effect of blotched coloration (Dijkstra P, personal communication). Social dominance might provide a selective advantage to blotched females, for example, in defending grazing grounds, such as patches of *aufwuchs*-covered rocks. Hence, individual variation in male mating preferences for female color morphs might be the expression of alternative tactics: males carrying male-rescue genes might be choosing superior (blotched) females without paying the costs of heavily biased offspring sex ratios. Males lacking rescue genes might avoid the costs of sex ratio distortion by mating with non-blotched females. We are now testing whether an association between male-rescue genes and male mating preferences for blotched coloration is present in a wild population of males.

An alternative explanation for the evolution of male mating preference variation in female color polymorphic species has been proposed recently by Lande et al. (2001). The authors suggested that, when there is intense male–male competition for females and a preexisting male preference for one color morph is present, males choosing “nonpreferred” females gain a selective advantage by incurring less competition for mating partners. If a male preference for nonblotched female coloration were found to be ancestral in *P. 'zebra gold'*, as shown for *N. omnicaeruleus* (Pierotti and Seehausen 2007), the origin of male preference polymorphism in this species might have been driven by the frequency-dependent selection envisaged by Lande et al. (2001).

We are only beginning to explore the complex interactions between natural and sexual selection in the “blotch” sex-linked color polymorphism that characterizes many of the cichlid species in several African lake radiations. Studies assessing the frequency distribution in the wild of individual male mating preference types and the relative fitness of different color morphs in different physical and social environments are needed to shed light onto the origin and maintenance of male mating preferences for female coloration in female polymorphic species and their possible role in cichlid fish adaptive radiation.

FUNDING

University of Hull PhD studentship to M.E.R.P.

We thank Russell Lande, Ola Svensson, Shinichi Nakagawa, and Charlie Cornwallis for helpful discussion and Alan Smith for assistance in

the aquarium. We would also like to thank Naomi Pierce and 3 anonymous referees for useful constructive comments on earlier drafts. This research adhered to the Association for the Study of Animal Behaviour/Animal Behavior Society Guidelines for the Use of Animals in Research, local legislation, and institutional code of conduct.

REFERENCES

- Albert AYK. 2005. Mate choice, sexual imprinting, and speciation: a test of a one-allele isolating mechanism in sympatric sticklebacks. *Evolution*. 59:927–931.
- Amundsen T, Forsgren E. 2001. Male mate choice selects for female colouration in a fish. *Proc Natl Acad Sci USA*. 98:13155–13160.
- Amundsen T, Forsgren E. 2003. Male preference for colourful females affected by male size in a marine fish. *Behav Ecol Sociobiol*. 54:55–64.
- Andersson M. 1994. *Sexual selection*. Princeton (NJ): Princeton University Press.
- Arnegard ME, Kondrashov AS. 2004. Sympatric speciation by sexual selection alone is unlikely. *Evolution*. 58:222–237.
- Baerends GP, Baerends-van Roon JM. 1950. An introduction to the study of the ethology of cichlid fishes. *Behaviour*. (Suppl 1): 1–242.
- Basolo AL, Delaney KJ. 2001. Male biases for male characteristics in females in *Priapella olmeca* and *Xiphophorus helleri* (family Poeciliidae). *Ethology*. 107:431–438.
- Bates D, Sarkar D. 2006. lme4: linear mixed-effects models using Eigen and syntax. R package version 0.99875-2. Available from: <http://cran.r-project.org>.
- Bonduriansky R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol Rev*. 76:305–339.
- Cohen J. 1988. *Statistical power analysis for the behavioral sciences*. Hillsdale (NJ): Erlbaum.
- Craig JK, Foote CJ. 2001. Countergradient variation and secondary sexual color: phenotypic convergence promotes genetic divergence in carotenoid use between sympatric anadromous and nonanadromous morphs of sockeye salmon (*Oncorhynchus nerka*). *Evolution*. 55:380–391.
- Darwin C. 1871. *The descent of man, and selection in relation to sex*. London: Murray.
- Genner MJ, Turner GF. 2005. The Mbuna cichlids of Lake Malawi: a model for rapid speciation and adaptive radiation. *Fish Fish*. 6:1–34.
- Grant PR, Grant BR. 1997. Hybridization, sexual imprinting, and mate choice. *Am Nat*. 149:1–28.
- Greene E, Lyon BE, Muehler VR, Ratcliffe L, Oliver SJ, Boag PT. 2000. Disruptive sexual selection for plumage coloration in a passerine bird. *Nature*. 407:1000–1003.
- Greenwood PH. 1974. The cichlid fishes of Lake Victoria, East Africa: the biology and evolution of a species flock. *Bull Br Mus (Nat Hist) Zool Suppl*. 6:1–134.
- Haesler MP, Seehausen O. 2005. Inheritance of female mating preference in a sympatric sibling species pair of Lake Victoria cichlids: implications for speciation. *Proc R Soc Lond B Biol Sci*. 272: 237–245.
- Hedeker D. 2005. Generalized linear mixed models. In: Everitt BS, Howell DC, editors. *Encyclopedia of statistics in behavioral science*. Vol. 2. Chichester (NH): Wiley. p. 729–738.
- Higashi M, Takimoto G, Yamamura N. 1999. Sympatric speciation by sexual selection. *Nature*. 402:523–526.
- Holzberg S. 1978. A field and laboratory study of the behaviour and ecology of *Pseudotropheus zebra* (Boulenger) an endemic cichlid of Lake Malawi (Pisces: Cichlidae). *Z Zool Syst Evolutionsforsch*. 16:171–187.
- Houde AE. 2001. Sex roles, ornaments, and evolutionary explanation. *Proc Natl Acad Sci USA*. 98:12857–12859.
- Huang CJ, Lin JY, Tsai HJ. 1999. Two distinct c-ski cDNAs of fish, tilapia (*Oreochromis aureus*). *Mol Reprod Dev*. 54:223–231.
- Jennions MD, Petrie M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biol Rev*. 72:283–327.
- Jordan R, Kellogg K, Juanes F, Stauffer J Jr. 2003. Evaluation of female mate choice cues in a group of Lake Malawi *Mbuna* (Cichlidae). *Copeia*. 1:181–186.
- Knight ME. 1999. *Sexual selection and speciation in Lake Malawi cichlids* [PhD dissertation]. Southampton (UK): University of Southampton.

- Knight ME, Turner GF. 1999. Reproductive isolation among closely related Lake Malawi cichlids: can males recognize conspecific females by visual cues? *Anim Behav.* 58:761–768.
- Kocher T. 2004. Adaptive evolution and explosive speciation: the cichlid fish model. *Nat Rev Genet.* 5:288–298.
- Konings A. 2001. Malawi cichlids in their natural habitat. 3rd ed. El Paso (TX): Cichlid Press.
- Kop PPAM, Heuts BA. 1973. An experiment on sibling imprinting in the jewel fish *Hemichromis bimaculatus* (Gill 1862, Cichlidae). *Rev Comp Anim.* 7:63–76.
- Lachlan RF, Servedio MR. 2004. Song learning accelerates allopatric speciation. *Evolution.* 58:2049–2063.
- Laland KN. 1994. On the evolutionary consequences of sexual imprinting. *Evolution.* 48:477–489.
- Lande R, Seehausen O, van Alphen JJM. 2001. Mechanisms of rapid sympatric speciation by sex reversal and sexual selection in cichlid fish. *Genetica.* 112–113:435–443.
- Lessells CM, Boag PT. 1987. Unrepeatable repeatabilities: a common mistake. *Auk.* 104:116–121.
- Mikami OK, Kohda M, Kawata M. 2004. A new hypothesis for species coexistence: male–male repulsion promotes coexistence of competing species. *Popul Ecol.* 46:213–217.
- Morris MR, Nicoletto PF, Hesselman E. 2003. A polymorphism in female preference for a polymorphic male trait in the swordtail fish *Xiphophorus cortezi*. *Anim Behav.* 65:45–52.
- Nakagawa S. 2004. A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behav Ecol.* 15:1044–1045.
- Pauers MJ, McKinnon JS, Ehlinger TJ. 2004. Directional sexual selection on chroma and within-pattern colour contrast in *Labeotropheus fuelleborni*. *Proc R Soc Lond B Biol Sci.* 271(Suppl 6):5444–5447.
- Pierotti MER, Seehausen O. 2007. Male mating preferences pre-date the origin of a female trait polymorphism in an incipient species complex of Lake Victoria cichlids. *J Evol Biol.* 20:240–248.
- Price T. 1998. Sexual selection and natural selection in bird speciation. *Philos Trans R Soc Lond B Biol Sci.* 353:251–260.
- Sappington TW, Taylor OR. 1990. Disruptive sexual selection in *Colias eurytheme* butterflies. *Proc Natl Acad Sci USA.* 87:6132–6135.
- Seehausen O, Schluter D. 2004. Male-male competition and nuptial colour displacement as a diversifying force in Lake Victoria cichlid fish. *Proc R Soc Lond B Biol Sci.* 271:1345–1353.
- Seehausen O, van Alphen JJM. 1998. The effect of male colouration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). *Behav Ecol Sociobiol.* 42:1–8.
- Seehausen O, van Alphen JJM, 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.
- Seehausen O, van Alphen JJM, Witte F. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science.* 277:1808–1811.
- Servedio MR, Lande R. 2006. Population genetic models of male and mutual mate choice. *Evolution.* 60:674–685.
- Siepen G, Crapon de Caprona MD. 1986. The influence of parental color morph on mate choice in the cichlid fish *Cichlasoma nigrofasciatum*. *Ethology.* 71:187–200.
- Sjölander S, Fernö A. 1973. Sexual imprinting on another species in a cichlid fish, *Haplochromis burtoni*. *Rev Comp Anim.* 7:77–81.
- Smithson M. 2003. Confidence intervals. London: Sage Publications.
- Sokal RR, Rohlf FJ. 1995. Biometry. New York: W.H. Freeman and Co.
- Stauffer JR, Bowers NJ, Kellogg KA, McKaye KR. 1997. A revision of the blue–black *Pseudotropheus zebra* (Teleostei: Cichlidae) complex from Lake Malawi, Africa, with a description of a new genus and species. *Proc Acad Nat Sci Phila.* 148:189–230.
- Streelman JT, Albertson RC, Kocher TD. 2003. Genome mapping of the orange blotch colour pattern in cichlid fishes. *Mol Ecol.* 12:2465–2471.
- Takimoto G, Higashi M, Yamamura N. 2000. A deterministic genetic model for sympatric speciation by sexual selection. *Evolution.* 54:1870–1881.
- Turner GF, Burrows MT. 1995. A model of sympatric speciation by sexual selection. *Proc R Soc Lond B Biol Sci.* 260:287–292.
- Verzijden MN, Lachlan RF, Servedio MR. 2005. Female mate-choice behavior and sympatric speciation. *Evolution.* 59:2097–2108.
- Wagner WE Jr. 1998. Measuring female mating preferences. *Anim Behav.* 55:1029–1042.
- Weber PG, Weber SP. 1976. The effect of female color, size, dominance and early experience upon mate selection in male convict cichlids, *Cichlasoma nigrofasciatum* Günther (Pisces, Cichlidae). *Behaviour.* 56:116–135.