

Male mating preferences pre-date the origin of a female trait polymorphism in an incipient species complex of Lake Victoria cichlids

M. E. R. PIEROTTI* & O. SEEHAUSEN†‡

*Department of Biological Sciences, Molecular and Evolutionary Ecology Group, University of Hull, Hull, UK

†Aquatic Ecology and Evolution, Institute of Zoology, University of Bern, Bern, Switzerland

‡EAWAG Ecology Research Center, Kastanienbaum, Switzerland

Keywords:

cichlid;
male choice;
pre-existing preference;
sex determination;
sexual selection;
sympatric speciation.

Abstract

Disruptive sexual selection on colour patterns has been suggested as a major cause of diversification in the cichlid species flock of Lake Victoria. In *Neochromis omnicaeruleus*, a colour and sex determination polymorphism is associated with a polymorphism in male and female mating preferences. Theoretical work on this incipient species complex found conditions for rapid sympatric speciation by selection on sex determination and sexual selection on male and female colour patterns, under restrictive assumptions. Here we test the biological plausibility of a key assumption of such models, namely, the existence of a male preference against a novel female colour morph before its appearance in the population. We show that most males in a population that lacks the colour polymorphism exhibit a strong mating preference against the novel female colour morph and that reinforcement is not a likely explanation for the origin of such male preferences. Our results show that a specific condition required for the combined action of selection on sex determination and sexual selection to drive sympatric speciation is biologically justified. Finally, we suggest that Lake Victoria cichlids might share an ancestral female recognition scheme, predisposing colour monomorphic populations/species to similar evolutionary pathways leading to divergence of colour morphs in sympatry.

Introduction

The explosive radiation of cichlid fishes in Lake Victoria provides ideal model systems to test the hypothesis that polymorphisms in mate preferences may cause strong premating isolation and rapid sympatric speciation with very small genome-wide differentiation. More than 500 haplochromine cichlid species have radiated from few ancestral populations within the past 100 000 years (Seehausen *et al.*, 2003; Verheyen *et al.*, 2003), and most of them perhaps within merely 14 600 years (Johnson *et al.*, 1996; Nagl *et al.*, 2000; Seehausen, 2002). Both in Lake Victoria and in Lake Malawi

cichlids, there is evidence that behavioural mate choice based on colour patterns is often the only isolating mechanism in sympatry (Holzberg, 1978; Marsh *et al.*, 1981; Seehausen *et al.*, 1997, 1998b; Knight *et al.*, 1998; Seehausen & van Alphen, 1998; Knight & Turner, 2004).

Several theoretical models have emphasized the potential for sexual selection to drive rapid allopatric (Lande, 1981; Kiestner *et al.*, 1984; Lande & Kirkpatrick, 1988; Iwasa & Pomiankowski, 1995; Gavrillets, 2000), parapatric (Lande, 1982; Kirkpatrick & Servedio, 1999; Kirkpatrick, 2000) or sympatric speciation (Wu, 1985; Turner & Burrows, 1995; Payne & Krakauer, 1997; van Doorn *et al.*, 1998, 2004; Higashi *et al.*, 1999; Kawata & Yoshimura, 2000; van Doorn & Weissing, 2001; Lande *et al.*, 2001; Gavrillets & Waxman, 2002; Takimoto *et al.*, 2000). However, empirical evidence is still scarce, and the generality and/or the biological plausibility of

Correspondence: Michele Pierotti, Molecular and Evolutionary Ecology Group, Department of Biological Sciences, University of Hull, Cottingham Road, HU6 7RX, Hull, UK.
Tel.: +44 (0)1482 466431; fax: +44 (0)1482 465458;
e-mail: m.pierotti@biosci.hull.ac.uk

conditions under which speciation is observed in such models is much debated (Turelli *et al.*, 2001; Kirkpatrick & Ravignè, 2002; Arnegard & Kondrashov, 2004; Kirkpatrick & Nuismer, 2004).

Lande *et al.* (2001) have proposed that multiple speciation events may occur when sex ratio selection and sexual selection by mutual mate choice interact. The authors model the invasion of a dominant female determiner in a male heterogametic system. This might be a relatively frequent scenario in highly structured populations where mild inbreeding favours female-biased population sex ratios (Bull, 1983; Werren *et al.*, 2002). The models of Lande *et al.* lead to the evolution of a new species carrying the dominant female determiner and an autosomal suppressor that restores the original male heterogamety. There is evidence that master sex determiners are not conserved across even closely related taxa (Saccone *et al.*, 2002; Volff *et al.*, 2003; Peichel *et al.*, 2004) and recent work suggests rapid evolution of new sex-determining systems by sequential upstream addition of new master switches to a relatively conserved regulatory pathway (Wilkins, 1995; Zarkower, 2001; Scharl, 2004).

A novel sexually selected trait physically linked to the female determiner could invade as it would be limited to females and therefore protected from sexual selection by female choice. In Lande *et al.* (2001), the novel trait acts as a marker for the dominant female gene and this triggers the evolution of individual male mating preferences for carriers of the dominant female determiner. Another appealing feature of these models of speciation by sexual selection is that genetic variation in mating preferences is not maintained by mutation or by external factors such as sudden shifts in the environment affecting sexual communication, as in most other models of sympatric speciation, but by means of disruptive selection (van Doorn *et al.*, 2004).

These speciation models were inspired by Seehausen *et al.* (1999a) study on the ecology and the genetics of colour and sex determination in *Neochromis omnicaeruleus*, a member of the rapidly speciating flock of Lake Victoria cichlids. In this species, some populations are polymorphic for male and female colorations and different colour morphs have identical microdistributions with no morphological or ecological differentiation (Seehausen & Bouton, 1997; Seehausen *et al.*, 1999a). Three colour morphs can occur sympatrically: (i) individuals exhibit dark vertical bars on the flanks, on a blueish (males) or a yellow-brown (females) background (P morph); alternatively, (ii) vertical bars are disrupted and appear as black blotches on a blueish (males) or white-yellow (females) background (WB morph); (iii) males and females exhibit black blotches on a pink to orange background (OB morph). Intermediate phenotypes exist, but are rare. Morph frequencies vary between populations and most populations lack the WB morph altogether. Similar male and female colour polymorphisms

have been observed among other species of Lakes Victoria, Kivu and Malawi (Snoeks, 1994; Konings, 1995; Seehausen, 1996; Seehausen *et al.*, 1999b; Lande *et al.*, 2001).

In *N. omnicaeruleus*, the genes associated with both blotched phenotypes (WB, OB) are linked to dominant female determiners (W) on the X chromosome. Their dominance over Y can be counteracted by autosomal suppressors (male rescue genes) in the absence of which female sex-biased clutches are produced (Seehausen *et al.*, 1999a). Blotched males are therefore carriers of both the blotch-linked sex ratio distorter and its autosomal suppressor. In colour polymorphic populations (e.g. Makobe Island population), males with the rescue gene were found to exhibit mate preferences for blotched females (WB/OB), whereas males without suppressor preferred females (P) lacking the dominant female determiner. The resulting selective mating among colour morphs led Seehausen *et al.* (1999a) to suggest that the *N. omnicaeruleus* polymorphism has properties of an incipient stage in sympatric speciation by sex ratio selection and disruptive sexual selection on male and female coloration.

Despite their potential to provide new insight into the role sex-linked genes and sex determination might play in the generation of new species, the models of Lande *et al.* (2001) rely on a restrictive assumption regarding the mate preference state of the ancestral population invaded by the novel sex-linked female colour. Counter-intuitively, a male mating preference against the novel female colour is required to be present before the appearance of the female colour in the population. Such pre-existing preference allows the creation of a pool of females of the novel colour avoided by the majority of males but available to rare mutant males that mate with such females sustaining less intense competition for mating opportunities. The resulting sexual selection on male mating preferences is necessary to obtain partial association between novel W-linked colour allele and novel male preference. However, this assumption may be problematic as there is no *a priori* reason to expect the presence of male mate choice in lekking species with highly skewed parental investment (Trivers, 1972; Parker, 1983; Kokko & Johnstone, 2002) such as the maternal mouthbrooding cichlids of Lake Victoria and Lake Malawi.

Male mating preferences against novel phenotypes in monomorphic populations may evolve to prevent hybridization with sympatric species (reinforcement; Dobzhansky, 1940; Noor, 1999). Alternatively, such male mating preferences could either represent an ancestral trait retained in a species that is a member of a recently radiated clade, or could have evolved by direct natural selection on mating preferences (Servodio, 2001; Albert & Schluter, 2004) or as a by-product of competition and ecological character displacement (Rundle & Schluter, 1998; Schluter, 2000, 2001).

Seehausen *et al.* (1999a) did find male preferences for and against the blotched female types in a colour polymorphic population of the Lake Victoria haplochromine *N. omnicaeruleus*; however, these might have evolved after the polymorphism appeared, e.g. under direct selection for genetic compatibility. Males susceptible to the sex ratio distorter would be selected to avoid mating with female carriers (blotched morphs WB and OB) and instead to prefer mating with the yellow-brown female morph (P); resistant males would preferably mate with female carriers (WB/OB). If this scenario is correct, we do not expect P monomorphic populations to harbour male mating preferences for or against P/WB female colour morphs.

Here we consider a colour monomorphic population (ancestral population in the model of Lande *et al.*) of the cichlid *N. omnicaeruleus*. We test whether there is any evidence of individual male mating preferences against (or for) a novel female colour morph prior to its occurrence in the population and whether any such preference could be the result of reinforcement of prezygotic reproductive isolation against sympatric closely related species. We find that most males show a strong mating preference against the novel female morph (WB), and that males that prefer the ancestral P over the novel WB females also prefer heterospecific P females over conspecific WB females. We conclude that a key assumption of Lande *et al.* (2001) models of sympatric speciation by selection on sex determination and mating preferences reflects real states of natural populations. We suggest that male Lake Victoria cichlids share a nonspecific ancestral female recognition scheme that might allow repeated generation of male mating preference polymorphisms in sympatry, in different populations and in different cichlid species, potentially leading to sympatric speciation, as modelled by Lande *et al.* (2001).

Materials and methods

Study species

We conducted our experiments on wild-caught *N. omnicaeruleus* males from the P monomorphic population of Ruti Island, Western Speke Gulf, Lake Victoria. Female pairs in conspecific trials and control males were wild-caught individuals from the colour polymorphic population of Makobe Island, which has been studied in the past for the presence of polymorphisms in male and female coloration, sex determination system and mate preferences (Seehausen *et al.*, 1999a). A WB morph has never been observed at Ruti Island among more than 400 fishes examined over more than a decade of sampling (Lande *et al.*, 2001, O. Seehausen, unpublished data collected between 2000 and 2004). In the Makobe Island population, more than 16% of the individuals belong to the WB morph, and Seehausen *et al.* (1999a) showed that males from that population are polymorphic for

individual male preferences for female colour morphs. As a control group for the Ruti Island sample (colour monomorphic population), males from the colour and male preference polymorphic population of Makobe Island, were tested with the same design.

For the conspecific/heterospecific trials, we used WB females from the Makobe Island colour polymorphic population of *N. omnicaeruleus* and P females from the Ruti Island colour monomorphic population of *Neochromis* 'yellow anal scraper' (Seehausen, 1996). *Neochromis* 'yellow anal scraper' differs very little in head morphology and body shape from *N. omnicaeruleus* but the two species have very distinct male nuptial coloration and coexist at several sites, including Ruti Island (Seehausen, 1996). Female *Neochromis* 'yellow anal scraper' are yellow-brown with dark vertical bars and very similar in colour pattern to *N. omnicaeruleus* females. If male mating preferences in *N. omnicaeruleus* for P females of their own species have evolved by reinforcement of reproductive isolation, males are expected to avoid mating with P females of the sympatric closely related species, *Neochromis* 'yellow anal scraper'.

Housing conditions

Males were collected at Ruti Island and Makobe Island, Southern Lake Victoria, and shipped to the laboratory in Hull where they were kept in large population tanks. Few months prior to the start of the experiment and for the entire duration of the experiment, males were kept in individual 19 × 19 × 18 cm aquaria allowing no visual contact with either females, or with heterospecifics with female-like colour patterns. Individual females were kept in transparent plastic enclosures inside larger tanks. The enclosures were identical to the ones used in the trials. All aquaria were maintained at 26 ± 2 °C and illuminated with daylight fluorescent bulbs 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.

Experimental design

We simultaneously presented *N. omnicaeruleus* males from the colour monomorphic population of Ruti Island with a yellow-brown (P) female and a black and white blotched (WB) female, both from the colour polymorphic population of Makobe Island. By using both female morphs from the same island, which is not the island the males came from, we control for possible 'own population' effects on male mating preferences. The number of lateral displays towards females was used as a measure of male courtship intensity. Lateral displays are widely used as the standard measure of cichlid courtship behaviour (e.g. Baerends & Baerends-van Roon, 1950; Carlstead, 1983; McElroy & Kornfield, 1990; Seehausen & van Alphen, 1998; Maan *et al.*, 2004). A standardized mean

preference score was calculated as the ratio (lateral displays to P female – lateral displays to WB female/total number of lateral displays).

The model of Lande *et al.* (2001) requires the presence of individual males with consistent male preferences for female coloration. Each experimental male was tested with six to eight different female pairs, and Wilcoxon signed rank tests were performed on the number of lateral displays to determine whether there were consistent individual preferences for either female colour morph. Wilcoxon signed rank tests on mean standardized lateral display scores of each male were used to compare direction and strength of male preferences between conspecific and heterospecific choice trials. No difference between female colour morphs was identified with respect to standard length, weight or gravidity stage in female pairs (Wilcoxon signed rank tests: all n.s.). We performed one-sample *t*-tests on the arcsin-transformed mean preference scores for the two separate male populations to test for deviation from the null expectation of no preference.

Mate choice trials

Individual males were given the choice between a P morph female and a WB morph female each placed in a transparent plastic enclosure at either end of a 100 × 40 × 40 cm experimental aquarium. Males were introduced into the experimental aquarium and left to acclimatize overnight. The following day, a yellow-brown barred female (morph P) and a blotched female (WB) were placed each in a plastic enclosure. For each trial a different set of females was used and every male was only tested once with each female. Males were left in the experimental tank between trials, and were tested with no more than three female pairs a day. The left and right positions of P and WB females were rotated randomly between trials. Male behaviour was video recorded and scored for 20 min from its first interaction with a female, defined as approaching the female to a distance of <20 cm. Trials in which the male did not interact with either female were discarded. After a trial, standard length (to the nearest 0.5 mm) and weight (to the nearest 0.1 g) were measured for each female. Gravidity stage was also assessed using a 1–5 score as follows: 1, immature/recently spent; 2, early ripening; 3, ripening; 4, fully ripe; 5, spent.

Results

We conducted 45 conspecific morph choice trials using seven wild-caught males from the colour monomorphic Ruti Island population (R1–R7) and 52 conspecific morph choice trials on seven wild-caught males of the colour polymorphic Makobe Island population (M1–M7).

When tested with a conspecific female colour morph pair (P vs. WB) from a polymorphic population (Makobe

Island; open bars in Fig. 1), six Ruti Island males had consistent mate preferences for the P over the WB female colour morph, one male (R1) did not show significant preferences, whereas none showed preference for females of the WB morph (Ruti Island males R1–R7: Wilcoxon signed rank tests: Table 1 and Fig. 1, shaded bars). An overall *P*-value, obtained from the individual Wilcoxon signed rank tests, using Fisher's combined probability test, was highly significant ($\chi^2_{14} = 43.110$; $P < 0.001$).

In contrast to the results for Ruti Island males and in line with a previous study (Seehausen *et al.*, 1999a), males from the colour polymorphic population (Makobe Island) exhibited individual variation in the direction of male mating preferences for female colour morph, with all three preference conditions, i.e. preference for P (male M1), preference for WB (males M3, M5–M7) and no preference (males M2, M4), represented (Wilcoxon signed rank tests: Table 1 and Fig. 1, open bars). Male preferences were significantly different from zero in the colour monomorphic Ruti population ($t = 6.054$, $P = 0.001$) but not significantly different from zero (i.e. no preference) in the colour polymorphic Makobe population ($t = -1.508$, $P = 0.182$).

To explore the possibility that reinforcement or reproductive character displacement is responsible for Ruti Island male preferences for the P female morph, we ran 12 conspecific/heterospecific choice trials on six Ruti Island males (R1–R5, R7), testing them with P females of the sympatric *Neochromis* 'yellow anal scraper' vs. conspecific *N. omnicaeruleus* WB females from the polymorphic population (Makobe Island). Ruti Island males preferred the heterospecific P female over the conspecific WB female. Male preferences in conspecific/heterospecific choice trials did not differ from those in conspecific choice trials (Wilcoxon signed rank test on mean preference scores: $n = 6$, $Z = -0.105$, $P = 0.917$; Fig. 2).

No significant differences were found in male courtship with regard to female size (standard length), weight or gravidity stage (Wilcoxon signed rank tests, all n.s.).

Discussion

Our results are consistent with the hypothesis that a male mating preference for P females in *N. omnicaeruleus* predates the origin of female colour polymorphism. We have shown that individual males in a colour monomorphic population (Ruti Island) have significant preferences against females of a novel colour. In contrast, males of a colour polymorphic population (Makobe Island) showed variation in male preferences for or against the alternative female colour morphs, confirming results of an earlier study (Seehausen *et al.*, 1999a). The novel male mating preference for females of the WB morph, observed in some males of the polymorphic population of Makobe Island is, therefore, likely to have evolved by selection on male mating preferences. There is evidence

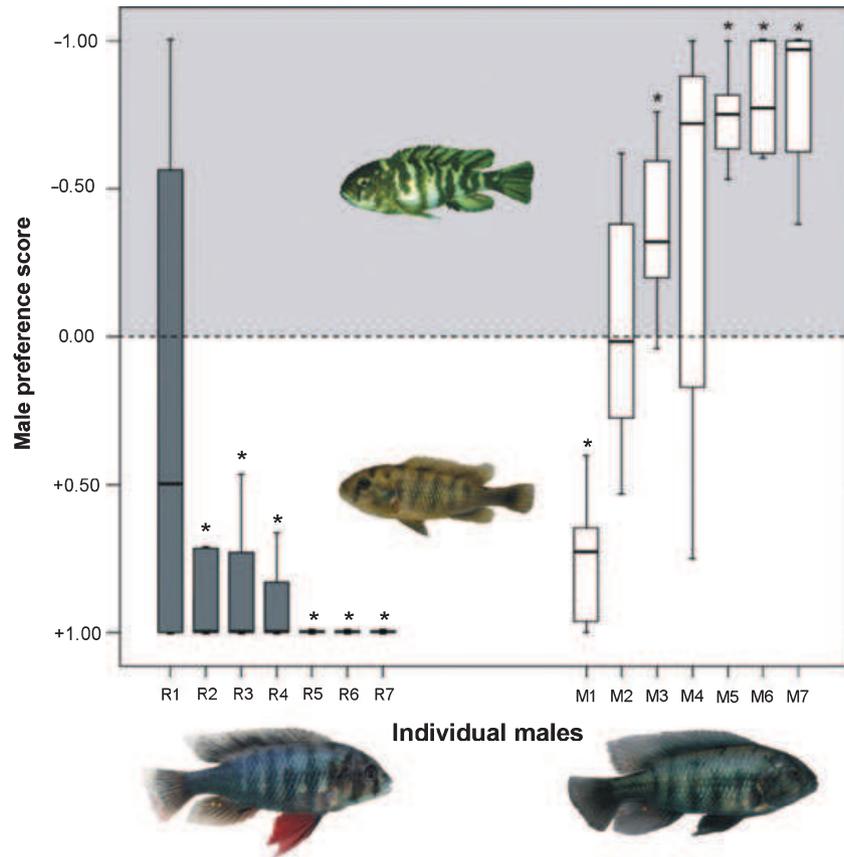


Fig. 1 Individual male preference scores [(lateral displays to P female – lateral displays to WB female)/total number of lateral displays]. Positive preference score values indicate male preference for P females, negative values indicate male preference for WB females. Shaded bars: wild-caught Ruti Island males (R1–R7); open bars: wild-caught Makobe Island males (M1–M7), both groups tested with P/WB Makobe Island females. Data are given as boxplot diagrams showing medians (middle line in the boxes), first and third quartiles (boxes) and range (whiskers). Significance levels are derived from *P*-values based on Wilcoxon signed-ranks tests on male choice trials: **P* < 0.05 (see also Table 1).

Table 1 *Z* and *P* values of Wilcoxon signed-ranks tests of male mating preference (number of courtship displays).

Male	Preference	<i>Z</i>	<i>P</i>	<i>N</i>
R1	No pref.	-1.05	0.29	6
R2	P	-1.99	0.046*	6
R3	P	-2.03	0.042*	8
R4	P	-2.21	0.027*	7
R5	P	-2.04	0.041*	6
R6	P	-2.21	0.027*	6
R7	P	-2.23	0.026*	6
M1	P	-2.201	0.028	6
M2	No pref.	-0.070	0.944	8
M3	WB	-2.197	0.028	7
M4	No pref.	-1.352	0.176	8
M5	WB	-2.371	0.018	7
M6	WB	-2.033	0.042	8
M7	WB	-2.524	0.012	8

Males R1–R7 are wild-caught individuals from the Ruti Island (monomorphic) population; males M1–M7 are wild-caught individuals from the Makobe Island (polymorphic) population.

that male mating preferences for female coloration have a genetic basis in the Makobe Island population of *N. omnicaruleus* (Seehausen *et al.*, 1999a; O. Seehausen & Y. Brandsen, unpublished) and in the Malawi cichlid

Pseudotropheus zebra 'gold' (M.E.R. Pierotti, unpublished). Consistent with the potential for male preferences to evolve, we observed variation in the strength of mating preference among the Ruti Island males. These results lend support to the speciation pathways envisaged by Lande *et al.* (2001), showing the biological plausibility of a critical model assumption, and suggesting that a condition of ancestral pre-existing male preferences might be a shared state among the rockdwelling cichlid species of Lake Victoria.

Males from Ruti Island use female coloration to identify potential partners: when given the choice between a conspecific blotched female (WB morph) from an allopatric population and a heterospecific yellow-brown female (P morph) from a sympatric species, males expressed a preference for the sympatric heterospecifics. Together with the fact that yellow-brown coloration is widespread in females of the haplochromine cichlid species flock of Lake Victoria (Greenwood, 1974, 1981; Seehausen, 1996) and represents most of the female fish community at Ruti Island, this suggests that male preferences are unlikely to be the product of reinforcement of reproductive isolation against closely related species. Instead it seems likely that a mating preference for a female colour that is shared among most species in Lake Victoria represents

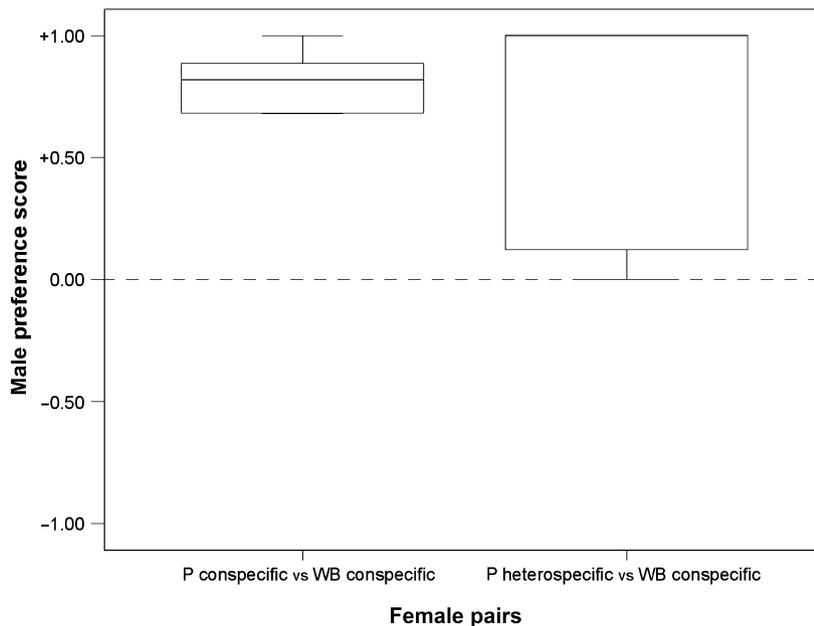


Fig. 2 Male preference scores in conspecific choice trials (*Neochromis omnicaeruleus* Makobe Is. females of P and WB colour morph) and in conspecific/heterospecific choice trials (*Neochromis* 'yellow anal scraper' Ruti Island females of P morph and *N. omnicaeruleus* Makobe Island females of WB morph). Data given as in Fig. 1.

an element of an ancestral mate recognition system. This may have evolved by direct selection or by reproductive character displacement against distantly related species prior to the adaptive radiation in Lake Victoria, or at least prior to the radiation of *Neochromis*. The riverine ancestors of the Lake Victoria cichlids lived in sympatry with other distantly related cichlids of the genera *Astatoreochromis*, *Pseudocrenilabrus* and *Oreochromis*. The females of these species differ in coloration, and hybrids between Lake Victoria cichlids (and most likely their common ancestor) and these cichlids are probably inviable (F. Witte, pers. comm. for *Astatoreochromis*; A. Smith, pers. comm. for *Oreochromis*) or at least infertile. Therefore, a preference against blotch may be the by-product of selection on mate recognition in the ancestral population that gave rise to the Lake Victoria radiation. By contrast, hybrids between species that belong to the radiation are fully fertile (Crapon de Caprona & Fritsch, 1984; Crapon de Caprona, 1986; Seehausen *et al.*, 1997), as is not surprising given the extreme young age of the radiation. This makes a recent origin by reinforcement of male mating preferences for yellow-brown females in *Neochromis* an implausible scenario. There is growing evidence for the effects of historical processes on mating preferences across speciation events (Ryan *et al.*, 2001). For example, in some *Drosophila* species female preferences for ancestral traits can persist in descendant populations where such preferences apparently lack any function (Kaneshiro, 1983). The gynogenetic fish *Poecilia formosa*, originated by hybridization about 10 000–100 000 years ago (Avise *et al.*, 1991), shows female preferences, in the absence of any male genetic contribution to the

offspring, that derive from its sexual parental species, *P. latipinna* and *P. mexicana* (Marler & Ryan, 1997).

An alternative explanation for the presence of male mating preferences in the *N. omnicaeruleus* monomorphic population of Ruti Island, involves effects of blotched coloration. A black and white blotched (WB) colour morph is not known for the Ruti Island population but an orange mottled morph, the heterozygous form of orange blotch (OB), was present in catches of the years 1991–1992 (0.6% frequency), but was never again seen in samples of several hundred females taken in the years 1993, 1995, 1996, 2000, 2002 and 2003 (O. Seehausen, unpublished). We do not know whether *N. omnicaeruleus* males distinguish between the different female base colours, i.e. yellow-brown (P morph), orange (OB morph), or yellow-whitish (WB morph), or whether they rely on the regular black bar pattern in P females as opposed to the disrupted pattern (blotched or mottled) in WB and OB females, or a combination of the two criteria. If orange-blotched and white-blotched colorations are equivalent for males, then the evolution of male preferences against WB females (i.e. for P females) could have been driven by the presence of an orange-blotched morph in this population in the past. In this scenario, a male preference against blotch could have evolved to avoid female-biased broods and perhaps, reduced clutch size due to the production of inviable YY individuals. Following the extinction of blotched morphs at Ruti Island, a preference for blotch would possibly be selected against. A male preference for plain females, on the other hand, would probably not be costly, and would more likely have been retained. Therefore, a past invasion/extinction of blotch would prepare the conditions for a

second invasion, this time triggering the evolutionary dynamics of Lande *et al.*, leading to rapid sympatric speciation.

Regardless of the historical path that led to the origin of male mating preferences in the Ruti Island population, our results indicate that the assumption of a pre-existing male mating preference, which is required for Lande *et al.* model of sympatric speciation by selection on sex reversal and sexual selection, is biologically realistic. If a blotch-linked dominant female determiner now invaded a population monomorphic for female coloration and male mating preferences such as the Ruti Island population we studied (e.g. by dispersal of blotched individuals from Makobe Island, 10 km away), we might witness the development of similar polymorphisms in sex determination, mate preferences and both male and female colour patterns, leading to assortative mating between colour morphs and potentially divergence in sympatry.

The brown-barred/blotch colour polymorphism is well represented in both Lake Victoria and Lake Malawi rockdwelling cichlid radiations (Greenwood, 1981; Konings, 1995; Seehausen, 1996) and a polymorphism in male mating preferences for either brown barred (P) or blotched (OB) female coloration has been found in the colour polymorphic cichlid *P. zebra* 'gold', a member of the Lake Malawi cichlid species flock (M.E.R. Pierotti *et al.*, unpublished). Knight & Turner (1999) showed that males of *P. zebra* 'gold' and of a sympatric closely related species were unable to distinguish conspecific from heterospecific females of the same colour morph, suggesting that reinforcement is unlikely to be responsible for male preference evolution in the *P. zebra* species complex. Although our experiment does not specifically address this issue, we suggest that male Lake Victoria (and possibly Lake Malawi) haplochromine cichlids might share a nonspecific ancestral female recognition scheme (brown with dark vertical bars). Future studies should specifically test this possibility: if confirmed, this would suggest that any population/species monomorphic for female coloration (and male mating preferences) might be prone to the evolution of male mating preference polymorphisms in sympatry, following invasion by blotch-linked female determiners, and might diverge in sympatry, as modelled by Lande *et al.* (2001). Moreover, although these models were inspired by a particular colour polymorphism, there is no reason why any other novel female trait, acting as a marker for a new sex determiner, could not trigger similar speciation dynamics.

There is growing evidence that sex ratio distorters may be more widespread than previously assumed (Hurst & Pomiankowski, 1998) and that the nature of the selfish elements involved is likely to determine whether male, female or mutual mate preferences evolve. Cytoplasmic factors are thought to be responsible for male mate choice in the butterfly *Acraea encedon* (Randerson *et al.*, 2000); sex ratio meiotic drive genes have shaped female preferences in stalk-eyed flies (Wilkinson *et al.*, 1998a,b;

Lande & Wilkinson, 1999); dominant X-linked female determiners have triggered the evolution of male and female mating preferences in the Lake Victoria cichlid fish *N. omnicaruleus*. The blotch polymorphism, widespread in the haplochromine cichlids of Lakes Victoria and Malawi (Kocher, 2004), can provide new insights in the interplay between sex ratio distorters, sexual selection, and the evolution of reproductive isolation. Perhaps this interplay is at the heart of rapid speciation events in the cichlid flocks of the East African Lakes.

Acknowledgments

We thank Martine Maan, John Mrosso, Mhoja Kayeba, Mohamed Haluna and Machuma Mumwisi Jongo for help in collecting the fish, the Tanzanian Fisheries Research Institute for research permits and the Ministry of Agriculture and Food Security for an export permission; Andy Gould and Alan Smith for their assistance with fish care, Russell Lande, Simone Immler, Dave Lunt, Africa Gomez, Cock van Oosterhout, and George Turner for fruitful discussions at various stages of the research and Felix Breden and two anonymous reviewers for valuable comments on the manuscript. This work was supported by a University of Hull PhD studentship to M.E.R.P.

References

- Albert, A.Y.K. & Schluter, D. 2004. Reproductive character displacement of male stickleback mate preference: reinforcement or direct selection? *Evolution* **58**: 1099–1107.
- Arnegard, M.E. & Kondrashov, A.S. 2004. Sympatric speciation by sexual selection alone is unlikely. *Evolution* **58**: 222–237.
- Avise, J.C., Trexler, J.C., Travis, J. & Nelson, W.S. 1991. *Poecilia mexicana* is the recent female parent of the unisexual fish *P. formosa*. *Evolution* **45**: 1530–1533.
- Baerends, G.P. & Baerends-van Roon, J.M. 1950. An introduction to the study of the ethology of cichlid fishes. *Behaviour Suppl.* **1**: 1–242.
- Bull, J.J. 1983. *Evolution of Sex Determining Mechanisms*. Benjamin/Cummings, Menlo Park, CA.
- Carlstead, K. 1983. Influences of motivation on display divergences in three cichlid fish species (*Haplochromis*). *Behaviour* **83**: 205–228.
- Crapon de Caprona, M.D. 1986. The use of fertile hybrids for the study of the accuracy of species recognition in cichlids. *Ann. Mus. R. Afr. Centr. Sci. Zool.* **251**: 117–120.
- Crapon de Caprona, M.D. & Fritzsche, B. 1984. Interspecific fertile hybrids of haplochromine cichlidae (Teleostei) and their possible importance for speciation. *Neth. J. Zool.* **34**: 503–538.
- Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. *Am. Nat.* **74**: 312–321.
- van Doorn, G.S. & Weissing, F.J. 2001. Ecological versus sexual selection models of sympatric speciation: a synthesis. *Selection* **2**: 17–40.
- van Doorn, G.S., Noest, A.J. & Hogeweg, P. 1998. Sympatric speciation and extinction driven by environment dependent sexual selection. *Proc. R. Soc. Lond. B Biol. Sci.* **265**: 1915–1919.

- van Doorn, G.S., Dieckmann, U. & Weissing, F.J. 2004. Sympatric speciation by sexual selection: a critical reevaluation. *Am. Nat.* **163**: 709–725.
- Gavrilets, S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. *Nature* **403**: 886–889.
- Gavrilets, S. & Waxman, D. 2002. Sympatric speciation by sexual conflict. *Proc. Natl. Acad. Sci. U.S.A.* **99**: 10533–10538.
- Greenwood, P.H. 1974. The cichlid fishes of Lake Victoria, East Africa: the biology and evolution of a species flock. *Bull. Br. Mus. Nat. Hist. (Zool.)* **6** (Suppl.): 1–134.
- Greenwood, P.H. 1981. *The Haplochromine Fishes of the East African Lakes*. Kraus International Publications, Munich.
- 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. Evol. Forsch.* **16**: 171–187.
- Hurst, L.D. & Pomiankowski, A. 1998. The eyes have it. *Nature* **391**: 223–224.
- Iwasa, Y. & Pomiankowski, A. 1995. Continual change in mate preferences. *Nature* **377**: 420–422.
- Johnson, T.C., Scholz, C.A., Talbot, M.R., Kelts, K., Ricketts, R.D., Ngobi, G., Beuning, K., Ssemmanda, I. & McGill, J.W. 1996. Late Pleistocene desiccation of Lake Victoria and rapid evolution of cichlid fishes. *Science* **273**: 1091–1093.
- Kaneshiro, K.Y. 1983. Sexual selection and direction of evolution in the biosystematics of Hawaiian Drosophilidae. *Ann. Rev. Entomol.* **28**: 161–278.
- Kawata, M. & Yoshimura, J. 2000. Speciation by sexual selection in hybridizing populations without viability selection. *Evol. Ecol. Res.* **2**: 897–909.
- Kiester, A.R., Lande, R. & Schemske, D.W. 1984. Models of coevolution and speciation in plants and their pollinators. *Am. Nat.* **124**: 220–243.
- Kirkpatrick, M. 2000. Reinforcement and divergence under assortative mating. *Proc. R. Soc. Lond. B Biol. Sci.* **267**: 1649–1655.
- Kirkpatrick, M. & Nuismer, S.L. 2004. Sexual selection can constrain sympatric speciation. *Proc. R. Soc. Lond. B Biol. Sci.* **271**: 687–693.
- Kirkpatrick, M. & Ravnigne, V. 2002. Speciation by natural and sexual selection: models and experiments. *Am. Nat.* **159**: S22–S35.
- Kirkpatrick, M. & Servedio, M.R. 1999. The reinforcement of mating preferences on an island. *Genetics* **151**: 865–884.
- Knight, M.E. & Turner, G.F. 1999. Reproductive isolation among closely related Lake Malawi cichlids: can males recognize conspecific females by visual cues? *Anim. Behav.* **58**: 761–768.
- 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. Lond. B Biol. Sci.* **271**: 675–680.
- Knight, M.E., Turner, G.F., Rico, C., van Oppen, M.J.H. & Hewitt, G.M. 1998. Microsatellite paternity analysis on captive Lake Malawi cichlids supports reproductive isolation by direct mate choice. *Mol. Ecol.* **7**: 1605–1610.
- Kocher, T.D. 2004. Adaptive evolution and explosive speciation: the cichlid fish model. *Nat. Rev. Genet.* **5**: 288–298.
- Kokko, H. & Johnstone, R. 2002. Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signalling. *Proc. R. Soc. Lond. B Biol. Sci.* **357**: 319–330.
- Konings, A. 1995. *Malawi Cichlids in their Natural Habitat*. Cichlid Press, Zevenhuizen, the Netherlands.
- Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proc. Natl. Acad. Sci. U.S.A.* **78**: 3721–3725.
- Lande, R. 1982. Rapid origin of sexual isolation and character divergence in a cline. *Evolution* **36**: 213–223.
- Lande, R. & Kirkpatrick, M. 1988. Ecological speciation by sexual selection. *J. Theor. Biol.* **133**: 85–98.
- Lande, R. & Wilkinson, G.S. 1999. Models of sex-ratio meiotic drive and sexual selection in stalk-eyed flies. *Genet. Res. Camb.* **74**: 245–253.
- Lande, R., Seehausen, O. & van Alphen, J.J.M. 2001. Mechanisms of rapid sympatric speciation by sex reversal and sexual selection in cichlid fish. *Genetica* **112**: 435–443.
- Maan, M., Seehausen, O., Söderberg, L., Johnson, L., Ripmester, 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. Lond. B Biol. Sci.* **271**: 2445–2452.
- Marler, C.A. & Ryan, M.J. 1997. Origin and maintenance of a female mating preference. *Evolution* **51**: 1244–1248.
- Marsh, A.C., Ribbink, A.J. & Marsh, B.A. 1981. Sibling species complexes in sympatric populations of *Petrotilapia trewavas* (Cichlidae, Lake Malawi). *Zool. J. Linn. Soc.* **71**: 253–264.
- McElroy, D.M. & Kornfield, I. 1990. Sexual selection, reproductive behavior and speciation in the mbuna species flock of Lake Malawi (Pisces: Cichlidae). *Environ. Biol. Fishes* **28**: 273–284.
- Nagl, S., Tichy, H., Mayer, W.E., Takezaki, N., Takahata, N. & Klein, J. 2000. The origin and age of haplochromine fishes in Lake Victoria, East Africa. *Proc. R. Soc. Lond. B Biol. Sci.* **267**: 1049–1061.
- Noor, M.A.F. 1999. Reinforcement and other consequences of sympatry. *Heredity* **83**: 503–508.
- Parker, G.A. 1983. Mate quality and mating decisions. In: *Mate Choice* (P. Bateson, ed.), pp. 141–166. Cambridge Univ. Press, Cambridge, UK.
- Payne, R.J.H. & Krakauer, D.C. 1997. Sexual selection, space and speciation. *Evolution* **51**: 1–9.
- Peichel, C.L., Ross, J.A., Matson, C.K., Dickson, M., Grimwood, J., Schmutz, J., Myers, R.M., Mori, S., Schluter, D. & Kingsley, D.M. 2004. The master sex-determination locus in threespine sticklebacks is on a nascent Y chromosome. *Curr. Biol.* **14**: 1416–1424.
- Randerson, J.P., Jiggins, F.M. & Hurst, L.D. 2000. Male killing can select for male mate choice: a novel solution to the paradox of the lek. *Proc. R. Soc. Lond. B Biol. Sci.* **267**: 867–874.
- Rundle, H.D. & Schluter, D. 1998. Reinforcement of stickleback mate preferences: sympatry breeds contempt. *Evolution* **52**: 200–208.
- Ryan, M.J., Phelps, S.M. & Rand, A.S. 2001. How evolutionary history shapes recognition mechanisms. *Trends Cogn. Sci.* **5**: 143–148.
- Saccone, G., Pane, A. & Polito, L.C. 2002. Sex determination in flies, fruitflies and butterflies. *Genetica* **116**: 15–23.
- Schartl, M. 2004. Sex chromosome evolution in non-mammalian vertebrates. *Curr. Opin. Gen. Dev.* **14**: 634–641.
- Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford Univ. Press, Oxford, UK.

- Schluter, D. 2001. Ecology and the origin of species. *Trends Ecol. Evol.* **16**: 372–380.
- Seehausen, O. 1996. *Lake Victoria Rock Cichlids: Taxonomy, Ecology and Distribution*. Verduyn Cichlids, Zevenhuizen, the Netherlands.
- Seehausen, O. 2002. Patterns in fish radiation are compatible with Pleistocene desiccation of Lake Victoria and 14 600 year history for its cichlid species flock. *Proc. R. Soc. Lond. B Biol. Sci.* **269**: 491–497.
- Seehausen, O. & van Alphen, J.J.M. 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. & Bouton, N. 1997. Microdistribution and fluctuations in niche overlap in a rocky shore cichlid community in Lake Victoria. *Ecol. Freshw. Fish.* **6**: 161–173.
- Seehausen, O., van Alphen, J.J.M. & Witte, F. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* **277**: 1808–1811.
- Seehausen, O., Witte, F., van Alphen, J.J.M. & Bouton, N. 1998b. Direct mate choice maintains diversity among sympatric cichlids in Lake Victoria. *J. Fish Biol.* **53** (Suppl. A): 37–55.
- Seehausen, O., van Alphen, J.J.M. & Lande, R. 1999a. Colour polymorphism and sex-ratio distortion in a cichlid fish as a transient stage in sympatric speciation by sexual selection. *Ecol. Lett.* **2**: 367–378.
- Seehausen, O., Mayhew, P.J. & van Alphen, J.J.M. 1999b. Evolution of colour patterns in East African cichlid fish. *J. Evol. Biol.* **12**: 514–534.
- Seehausen, O., Koetsier, E., Schneider, M.V., Chapman, L.J., Chapman, C.A., Knight, M.E., Turner, G.F., van Alphen, J.J.M. & Bills, R. 2003. Nuclear markers reveal unexpected genetic variation and a Congolese-Nilotic origin of the Lake Victoria cichlid species flock. *Proc. R. Soc. Lond. B Biol. Sci.* **270**: 129–137.
- Servedio, M.R. 2001. Beyond reinforcement: the evolution of premating isolation by direct selection on preferences and postmating, prezygotic incompatibilities. *Evolution* **55**: 1909–1920.
- Snoeks, J. 1994. The haplochromines (Teleostei, Cichlidae) of Lake Kivu (East Africa). *Ann. Mus. R. Afr. Centr. Sci. Zool.* **270**: 1–221.
- Takimoto, G., Higashi, M. & Yamamura, N. 2000. A deterministic genetic model for sympatric speciation by sexual selection. *Evolution* **54**: 1870–1881.
- Trivers, R. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man 1871–1971* (B. Campbell, ed.), pp. 139–179. Aldine Press, Chicago, IL.
- Turelli, M., Barton, N.H. & Coyne, J.A. 2001. Theory and speciation. *Trends Ecol. Evol.* **16**: 330–343.
- Turner, G.F. & Burrows, M.T. 1995. A model of sympatric speciation by sexual selection. *Proc. R. Soc. Lond. B Biol. Sci.* **260**: 287–292.
- Verheyen, E., Salzburger, W., Snoeks, J. & Meyer, A. 2003. Origin of the superflock of cichlid fishes from Lake Victoria, East Africa. *Science* **300**: 325–329.
- Volf, J.-N., Kondo, M. & Schartl, M. 2003. Medaka dmy/dmrt1Y is not the universal primary sex-determining gene in fish. *Trends Genet.* **19**: 196–199.
- Werren, J.H., Hatcher, M.J. & Godfray, H.C.J. 2002. Maternal-offspring conflict leads to the evolution of dominant zygotic sex determination. *Heredity* **88**: 102–111.
- Wilkins, A.S. 1995. Moving up the hierarchy: a hypothesis on the evolution of a genetic sex determination pathway. *Bioessays* **17**: 71–77.
- Wilkinson, G.S., Presgraves, D.C. & Crymes, L. 1998a. Male eye span in stalk-eyed flies indicates genetic quality by meiotic drive suppression. *Nature* **391**: 276–279.
- Wilkinson, G.S., Kahler, H. & Baker, R.H. 1998b. Evolution of female mating preferences in stalk-eyed flies. *Behav. Ecol.* **9**: 525–533.
- Wu, C.-I. 1985. A stochastic simulation study on speciation by sexual selection. *Evolution* **39**: 66–82.
- Zarkower, D. 2001. Establishing sexual dimorphism: conservation amidst diversity? *Nat. Rev. Genet.* **2**: 175–185.

Received 1 April 2006; revised 15 June 2006; accepted 3 July 2006