

Intraspecific sexual selection on a speciation trait, male coloration, in the Lake Victoria cichlid *Pundamilia nyererei*

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The haplochromine cichlids of Lake Victoria constitute a classical example of explosive speciation. Extensive intra- and interspecific variation in male nuptial coloration and female mating preferences, in the absence of postzygotic isolation between species, has inspired the hypothesis that sexual selection has been a driving force in the origin of this species flock. This hypothesis rests on the premise that the phenotypic traits that underlie behavioural reproductive isolation between sister species diverged under sexual selection within a species. We test this premise in a Lake Victoria cichlid, by using laboratory experiments and field observations. We report that a male colour trait, which has previously been shown to be important for behavioural reproductive isolation between this species and a close relative, is under directional sexual selection by female mate choice within this species. This is consistent with the hypothesis that female choice has driven the divergence in male coloration between the two species. We also find that male territoriality is vital for male reproductive success and that multiple mating by females is common.

Keywords: colour evolution; cichlid fish; mate choice; *Pundamilia nyererei*; sexual selection; speciation

1. INTRODUCTION

The role of selection in the origin of new species is a relatively poorly understood problem in evolutionary biology and theoretical studies greatly outnumber experimental ones (Kirkpatrick & Ravigne 2002). However, without empirical data on natural populations, it is impossible to judge how realistic model assumptions are. The cichlid fish species flocks of the Great Lakes of East Africa, and the haplochromine radiations in Lakes Victoria and Malawi in particular, are classic examples of rapid speciation and adaptive radiation within the confines of single lakes. Lake Victoria is the youngest of the African Great Lakes (0.25–0.75 Myr; Fryer 1996) and sedimentological and palaeoclimatic evidence indicates that it was completely dry as recently as 14 500 years ago (Johnson *et al.* 1996, 2000). Although the genetic diversity contained in the flock must be far older (Nagl *et al.* 2000), most of the 500 or more endemic species must have diverged in an extremely short time. Several different models of speciation involving selection on mate-choice traits have been proposed to explain this (Kocher 2004). Theoretically, mate choice can

establish reproductive isolation very rapidly, since it directly influences mating behaviour and does not require postzygotic reinforcement through ecological selection against hybrids (Kirkpatrick & Ravigne 2002). Empirical support for sexual selection as a diversifying force has been demonstrated in birds (Barraclough *et al.* 1995; Uy & Borgia 2000; Irwin *et al.* 2001), lizards (Stuart-Fox & Owens 2003), parrotfishes (Streelman *et al.* 2002), insects (Arnqvist *et al.* 2000; Gray & Cade 2000), spiders (Masta & Maddison 2002), and snails (Schilthuizen 2003) but the evidence is not always unambiguous (e.g. Boake *et al.* 1997; Houde & Hankes 1997; Gage *et al.* 2002).

The appearance of, and subsequent frequent changes in, male nuptial coloration in East African cichlids is associated with the evolution of polygynous mating systems that would allow for stronger sexual selection (Seehausen *et al.* 1999a). Despite the morphological diversity that evolved in adaptive radiations of cichlids, closely related species of haplochromines typically have very similar morphologies and ecologies but differ strikingly in coloration (Albertson *et al.* 1999; Seehausen & Van Alphen 1999; Allender *et al.* 2003), with this variation affecting interspecific mate choice (Seehausen & Van Alphen 1998; Van Oppen *et al.* 1998).

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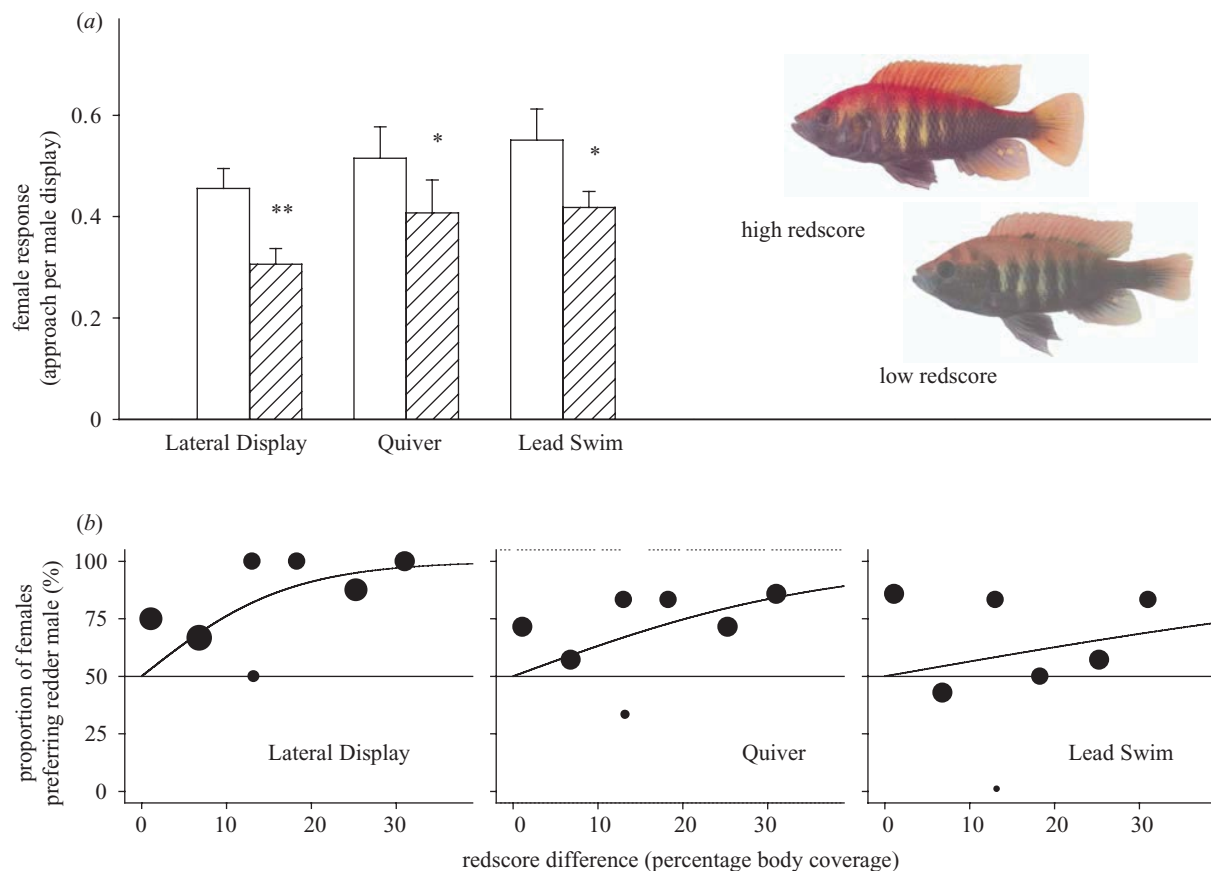


Figure 1. Mate-choice experiment in the laboratory. (a) Mean and standard error of female response to males with high (open bars) and low (hatched bars) redscores. Data are pooled over all trials for each female. (b) Probability curves for female preference depending on the difference in redscore between two males. For each male pair in the experiment, circles represent the proportion of females that preferred the redder male. Symbol sizes indicate sample sizes. Curves are forced through the origin.

However, interspecific differences in characters subject to mate choice do not necessarily signify that sexual selection was the cause of their divergence. Additional support must come from studies that specifically test whether traits responsible for reproductive isolation *between* sister species evolve under sexual selection *within* species (Lande 1981; West-Eberhard 1983; Boake 2002). Such evidence is rare. One possible example is a North American butterfly in which intraspecific female mate choice using male wing melanization is responsible for behavioural reproductive isolation between species (Wiernasz & Kingsolver 1992). We report experiments and field studies that constitute an explicit test of this prediction of the speciation-by-sexual-selection hypothesis in haplochromine cichlids.

Pundamilia pundamilia (Seehausen *et al.* 1998) and *Pundamilia nyererei* (Witte-Maas & Witte 1985) are rock-dwelling haplochromine cichlids endemic to Lake Victoria. *Pundamilia nyererei* is sympatric with *P. pundamilia* in all of its range; the two are very closely related and exchange genes in some localities (Seehausen 1996; Taylor *et al.* unpublished data). They differ only slightly in anatomy and both species exhibit vertical melanin bars that are more pronounced in males than in females. However, whereas females of both species are cryptically coloured and can be distinguished only with difficulty, *P. pundamilia* males are metallic blue-grey and *P. nyererei* males are bright red dorsally and yellow laterally. This difference in male colour

pattern is important in female mate choice: in laboratory experiments, females choose conspecific males when colours are visible, but not when they are masked (Seehausen & Van Alphen 1998).

We test within *P. nyererei* whether females select for brighter red males, using three approaches. First, we conduct mate-choice experiments in the laboratory to test whether females prefer the redder one of two males, and whether the strength of preference increases with the magnitude of the difference in redness. Second, we investigate the importance of male coloration as a predictor of female responsiveness in nature, where female choice could also be influenced by extended phenotype traits and other sources of variation. Third, we test whether male coloration predicts male mating success in a mesocosm experiment.

2. MATERIAL AND METHODS

(a) Fishes

We studied a *P. nyererei* population at Makobe Island in the western Speke Gulf (Tanzania; Seehausen & Bouton 1997), where the water is relatively clear (Secchi reading mean \pm s.e.m. = 221 ± 15 cm in the study period). All experiments and observations were carried out in 2001. Mature *P. nyererei* males defend territories on rocky bottoms at 4–7 m water depth. They attract females by vigorous courtship displays that resemble those of other haplochromine species (Seehausen & Van Alphen 1998). Courtship typically starts with Lateral Display, in which the male positions

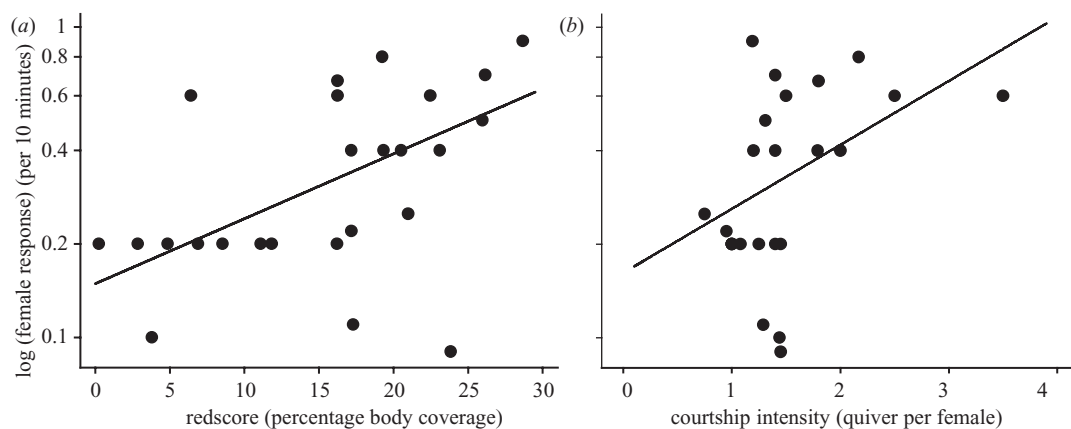


Figure 2. Field observations: the effects of male redscore (a) and male courtship intensity (b) on female response.

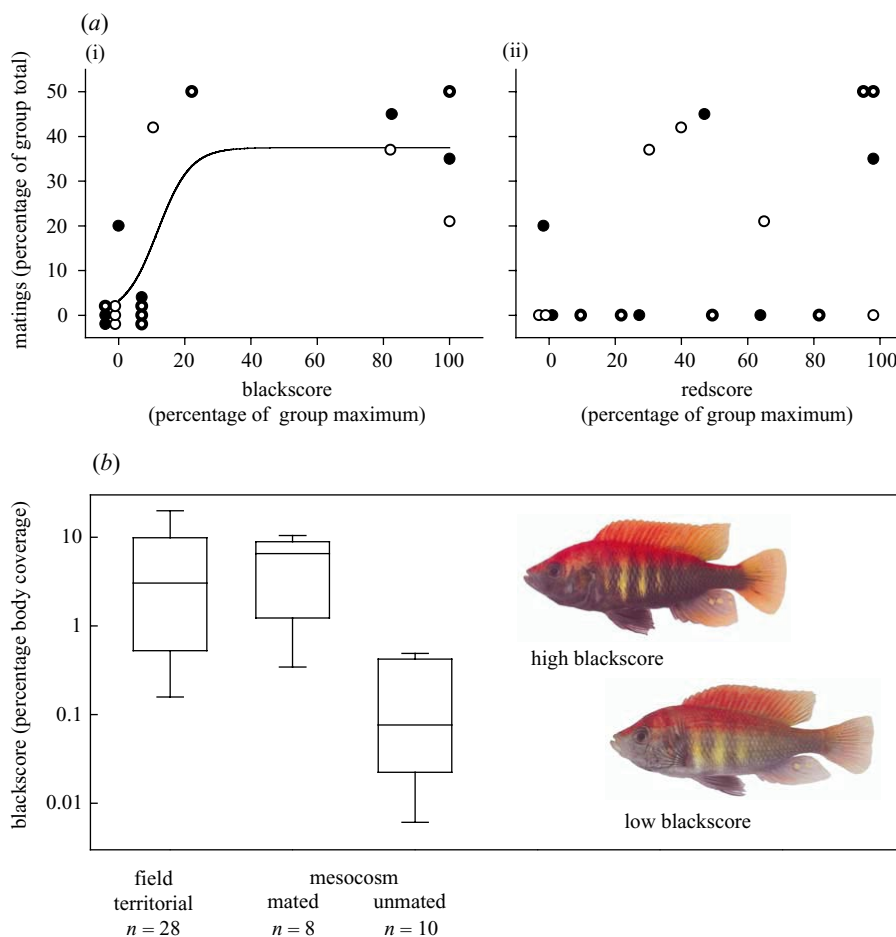


Figure 3. Mesocosm experiment. (a) Male blackscore (i) and redscore (ii) and the number of matings in each group: filled circles, group 1; open circles, group 2; half-open circles, group 3. (b) Blackscores of territorial males in the field and of mated and unmated males in the mesocosm experiment. Boxes represent 75% confidence intervals intercepted by the median; error bars represent 95% confidence intervals.

itself perpendicular to the female and spreads all fins. This is followed by Quiver, a high-frequency shaking movement of the body. Finally, the male leads the female to the centre of the territory in a quick swimming bout, often with exaggerated tail beats (Lead Swim). Mating occurs in rocky crevices, and immediately after spawning the mouthbrooding female leaves the territory. Females mouthbrood eggs and larvae for approximately three weeks, and guard the fry for an additional week after releasing them (Seehausen 1996).

(b) Mate-choice experiment in the laboratory

Two-way mate-choice experiments were performed following Seehausen & Van Alphen (1998): each male was kept in one of two six-sided Perspex enclosures (140 l) on either side of an 1800 l aquarium. In each enclosure, a brick on top of a PVC tube provided shelter. These shelters were readily accepted by the males as the centres of their territories. In the middle of the main tank two large stones provided shelter for the test female and ensured that the males could not see each other. The water in the male

enclosures was filtered internally, making chemical communication impossible. Air stones were present in both enclosures and in the main tank; water temperature was kept at 24–26 °C.

We used 25 wild-caught *P. nyererei*: 14 males, assembled into seven male pairs of fixed composition, and 11 females. All males were photographed, measured and weighed. Pairs were assembled such that the redscore differences between males in a pair varied from 1 to 30% body coverage. Size differences between paired males were small and did not covary with colour differences (standard length (SL): high redscore: 95.3 ± 5.7 mm, low redscore: 95.9 ± 4.5 mm (paired *t*-test: $t_6 = -0.54$, $p = 0.61$); weight: high redscore: 27.3 ± 7.3 g, low redscore: 27.0 ± 3.0 g (paired *t*-test: $t_6 = 0.17$, $p = 0.87$)).

Males were released into their compartments the evening before trials. A maximum of three females was tested with one male pair in a day and at least 2 h separated consecutive trials. Male pairs were exchanged after 3 successful trials or after 2 days. The position of the males (left or right side of the aquarium) was reversed each time the same pair was introduced. To start a trial, the test female was released in the middle of the tank. Behaviour was recorded with Observer 3.0 software (Noldus). Observation time started when the test female was within 30 cm of either one of the male compartments and stopped when she left this area. This distance was chosen because, in field observations, any fish within 30 cm of a *P. nyererei* territory elicits a behavioural response of the territory owner, indicating 30 cm as an interaction threshold distance. Trials were completed after 15 min of observation time.

Each female was tested once with every male pair, but in the analysis we included only those trials in which both males courted (performing Quiver or Lead Swim at least once) and the test female responded positively (approaching a male in response to his Lateral Display, Quiver or Lead Swim) to at least one of them (48 trials). For every male courtship display event, we recorded whether the female responded by approaching the male. The resulting proportion is our measure of female response to each male. The difference in female response to two males in a trial is our measure of female preference. We also recorded aggressive behaviour of the males (butting and biting attempts) and counted the total number of encounters of each male with the test female. Courtship intensity was defined as the mean number of courtship behaviours per encounter with the female and was calculated separately for Lateral Display, Quiver and Lead Swim.

(c) Field observations

We studied 28 territorial males, located between 4 and 7 m water depth, individually recognizable by their coloration pattern and/or injuries or scars. Using SCUBA, we collected between 5 and 10 focal observations of 10 min duration each for each male, yielding a total of between 50 and 100 min observation time per male (total observation time: 1950 min). Observations were carried out between 09.00 and 13.00 and all observations for one male were completed within a period of 6–32 days. We recorded all interactions between these males and conspecific females. Female response was defined as the proportion of courted females responding positively (i.e. approaching the male) to male courtship. Aggressive behaviour towards females, including aggressive Lateral Display (interpreted as such when it was followed by Frontal Display or chasing or biting), was much more common in these observations than in the laboratory experiments. We therefore used the number of times a male quivered to a female as our measure of male courtship intensity. Quiver is only rarely displayed in antagonistic contexts, unless the antagonists are of similar size and the antagonism escalates into a fight. This would

never be the case in interactions between territorial males and females in nature (SL of territorial males: 80.9 ± 0.5 mm ($n = 28$); random sample of 45 adult females: 63.5 ± 0.4 mm). We measured territory size (in square metres) of each male; territory borders were determined by the aggressive behaviour that the owner showed towards other fish (con- and heterospecific). After completion of behavioural data collection, all males were caught either in gillnets or by hook and line, both using SCUBA. Immediately thereafter males were measured (SL; to the nearest 0.1 mm) and photographed for colour analysis.

(d) Mesocosm experiment

We built three outdoor concrete ponds of ca. 3 m × 2 m × 1 m (length × width × depth) each at the Mwanza station of the Tanzania Fisheries Research Institute (TAFIRI), on the eastern shore of the Mwanza Gulf. After collection from Makobe Island, fish were kept in groups of approximately 40 individuals in larger ponds for three weeks. After this acclimatization period we fin-clipped all fish, photographed the males and assembled three groups of six adult males and 20 adult females that were released in each pond. Twenty PVC tubes (length 15 cm, diameter 10 cm) in each pond provided shelter. For a period of three weeks, starting one week after releasing the fish, we collected the eggs of every brooding female (10, 11 and 7 clutches for groups 1, 2 and 3). To do so, ponds were seined once a week. Average clutch size was 48 ± 5 eggs; all eggs were preserved in absolute ethanol. We used the number of matings, i.e. the number of clutches completely or partly sired by a male, as a measure of male reproductive success.

DNA was extracted from 10 offspring of each clutch using a Nucleon BACC2 kit according to manufacturer's protocol (Amersham). DNA was precipitated in ice-cold absolute ethanol, washed in 70% ethanol, air-dried for 20 min and dissolved in 30 µl of sterilized water. After checking the extraction on a 0.8% agarose gel, DNA concentration was determined by spectrometry. We used four tetranucleotide microsatellite loci for paternity assignment (Ppun5, Ppun7, Ppun20, Ppun21; Taylor *et al.* 2002). For each sample, ca. 25 ng of DNA, diluted in 1 µl of MilliQ, was added to a PCR mixture containing 4.648 µl of MilliQ, 0.2 mM of each dNTP (Promega), 5 pmol of both forward- and reverse primer, 0.26 U Taq polymerase (Bioline), 2.0 mM of Mg Cl₂ and 1 µl of 10× Mg free reaction buffer (Bioline). PCR programmes were: Ppun5: two-step protocol: 3 min at 94 °C, followed by five cycles of 94 °C for 30 s, 62 °C for 30 s and 72 °C for 30 s followed by 30 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, followed by one cycle of 10 min of 72 °C. Ppun7: standard protocol: 3 min at 94 °C, followed by 30 cycles of 94 °C for 30 s, 57 °C for 30 s and 72 °C for 30 s, followed by one cycle of 10 min of 72 °C. Ppun20 and Ppun21: touch down protocol: 3 min at 94 °C, followed by eight cycles of 94 °C for 30 s and 60 °C dropping one degree every cycle (down to 53 °C) for 30 s and 72 °C for 30 s, followed by 25 cycles of 94 °C for 30 s, 53 °C for 30 s and 72 °C for 30 s, followed by one cycle of 10 min of 72 °C.

The result of the PCR reaction was checked on a 1.5% agarose gel together with a 100 bp ladder. Amplification products were resolved on an ABI 377 sequencer, processed in GeneScan 3.1 and scored in Genotyper 3.1 (Perkin-Elmer). Of 260 analysed offspring, 13 were discarded because of insufficient DNA. Exclusion probability was 99.8% and all males were excluded as sires except one for all remaining 247 larvae (in 28 clutches; 8.8 ± 0.3 offspring per clutch).

(e) Colour analysis

Males were photographed under standard conditions, placed in a Perspex cuvette with water and gently squeezed between a grey PVC sheet and the front window. We used a single lens reflex camera with 100 mm lens and one flash on either side. All pictures were digitized. In PhotoShop 6.0 (Adobe Systems Inc.) we adjusted white balance with the aid of a white patch that was attached to the front side of the cuvette. We analysed the colours of the fish body, excluding fins and eyes, in SigmaScan Pro 4.0 (SPSS Inc.). To calculate colour scores, we defined criteria to delimit the body area covered by 'red' and 'yellow' by a combination of hue and saturation (red: hue = 0–26 plus 232–255, saturation 40–97%; yellow: hue = 27–45, saturation 40–97%) and subsequently calculated the area of the fish body that matched these criteria, yielding a percentage of body coverage. We similarly defined criteria for 'blackness', to quantify the body coverage of the black vertical bars and ventral aspects of the body (black: intensity = 0–75).

(f) Statistics

Comparisons of groups and bivariate relationships were analysed by using paired *t*-tests and Pearson correlations for normally distributed data, and Wilcoxon signed ranks tests (for dependent samples), Mann–Whitney *U*-tests (for independent samples) and Spearman's correlations for non-normally distributed data (SPSS 10.0 (SPSS Inc.)). Means of normally distributed data are given with standard errors.

For the mate-choice experiments in the laboratory, we analysed the influences of male size and colour on female response on a continuous scale using models with a binomial distribution and a logit-link function. We subsequently calculated probability curves for female preference using the same method. Reproductive success of males in the mesocosm experiment was expressed as a proportion of the total number of matings in each group and therefore also analysed using binomial models. Female response to male characteristics in the field were recorded as counts and analysed using models with a Poisson distribution and a log-link function. All generalized linear models (GLMs) were calculated in R (Ihaka & Gentleman 1996; <http://www.r-project.org>). Stepwise removal of non-significant variables from saturated models yielded minimal adequate models; significance was determined by *F*-tests examining the change in deviance after removal of each variable. We checked for over- and underdispersion and adjusted test statistics (Venables & Ripley 2002).

3. RESULTS

(a) Mate-choice experiment in the laboratory

Females responded stronger to the courtship of males with high redscores (paired *t*-tests comparing average response to both males over all trials per female: Lateral Display $t_{10} = 3.88$, $p = 0.003$; Quiver $t_{10} = 3.08$, $p = 0.012$; Lead Swim $t_9 = 2.30$, $p = 0.047$; figure 1a). To test the relative importance of male coloration and size we analysed female preference using GLMs with a binomial distribution, with logit-transformed female response as a dependent variable and male coloration (red, yellow and black), SL and weight as independent variables. Only male redscore significantly influenced female response to male Lateral Display (estimate = 4.25 ± 0.79 , $F_{1,47} = 35.13$, $p < 0.0001$) and Lead Swim (estimate = 3.44 ± 1.05 , $F_{1,43} = 13.29$, $p < 0.001$). Female response to male Quiver was also best explained by male redscore (estimate = 4.98 ± 1.11 , $F_{1,46} = 10.93$, $p < 0.002$) but significantly decreased with male blackscore (estimate = -1.51 ± 0.65 , $F_{1,46} =$

11.78 , $p < 0.002$). Besides the variation explained by covariates, there was no significant variation in response between females or between male pairs. Female responsiveness did not increase with the redness means of males in a pair (Spearman's rank correlations: $n = 7$, $p > 0.7$), confirming that it was the difference in redscore between the two males rather than the absolute redscore of the preferred male that explained female response.

Males with high and low redscores did not differ in courtship behaviour, aggression or encounter rates with females (Wilcoxon signed-ranks tests on medians of trials per male: $n = 7$ male pairs, $Z < 1.19$, $p > 0.24$) and courtship intensity was not correlated with redscore (Spearman's rank correlation of medians of trials per male: $n = 14$ males; $p > 0.35$ for each of the courtship behaviours). Female preferences were not correlated with behavioural differences between the males ($n = 48$ trials; $p > 0.45$).

To define the threshold above which females start discriminating between male redscores, we performed a GLM analysis with the difference in redscore as the single explanatory variable and the number of females preferring either male as binomially distributed dependent variable. Figure 1b reports the predicted proportion of females preferring the reddest male in a pair, for any given difference in redscore between them (estimate(Lateral Display) = 0.12 ± 0.036 , $F_{1,47} = 20.56$, $p = 0.004$; estimate(Quiver) = 0.054 ± 0.017 , $F_{1,43} = 13.12$, $p = 0.011$, estimate(Lead Swim) = 0.026 ± 0.022 , $F_{1,39} = 1.42$, $p = 0.29$). The curves become less steep as the escalation of courtship behaviours proceeds, and the curve for Lead Swim is not significant. This could be a sample size effect since not all courtship bouts proceeded to higher escalation levels, resulting in decreasing numbers of trials that met calculation criteria in the sequence Lateral Display–Quiver–Lead Swim; and decreasing numbers of observations within trials in the same sequence. For Lateral Display, calculation criteria were met in all 48 trials (Lateral Display frequency 31.7 ± 4.1 per male per trial), for Quiver in 44 trials (23.9 ± 3.6) and for Lead Swim in 40 trials (18.8 ± 3.1). The curves for Lateral Display and Quiver, both significant, indicate that females started discriminating as soon as there was a difference in redscore between the males.

(b) Reproductive success in nature

Female response to male courtship was analysed in a GLM including male territory size, water depth, male SL, redscore, blackscore, yellowscore and courtship intensity as independent variables and female response to male courtship as dependent variable. Male redscore and courtship intensity significantly explained female response (estimate (redscore) = 0.046 ± 0.016 , $F_{1,26} = 10.25$, $p = 0.004$; estimate(courtship intensity) = 0.415 ± 0.142 , $F_{1,25} = 7.66$, $p = 0.011$; figure 2).

These effects were independent: redder males did not court more intensely (Pearson correlation, $n = 27$ (one male did not court), $r = 0.16$, $p = 0.44$). The redscores of the males ranged from 0.3 to 28.7% body coverage ($15.4 \pm 1.5\%$); a range similar to that among the males used in the laboratory experiment ($3.3–34.3\%$; $18.4 \pm 2.8\%$).

There was a significant interaction between territory size and depth. Territories were clustered in two depth categories, with 11 males at 4.2 ± 0.1 m depth and 17 males at 6.3 ± 0.1 m depth. Territories in shallow water were larger

Table 1. Summary of the results of the mesocosm experiment.

	group		
	1	2	3
number of mated males	3	3	2
number of clutches	10	11	7
number of genotyped fry	89	92	69
number of clutches with:			
one father (% of clutches)	3 (30)	4 (36)	2 (29)
two fathers (% of clutches)	4 (40)	6 (55)	5 (71)
three fathers (% of clutches)	3 (30)	1 (9)	0 (0)
total multiple			
paternity (% of clutches)	7 (70)	7 (64)	5 (71)

than those in deeper water (4.14 ± 0.81 and $2.03 \pm 0.36 \text{ m}^2$; $t_{26} = 2.70$, $p = 0.012$). Depth did not influence female choice, and redscore and courtship intensity did not correlate with depth or territory size. However, female choice for high redscore and courtship intensity also selected for males with territories that were large relative to others at the same depth (GLM explaining female response by depth and territory size: estimate(territory size) = 0.137 ± 0.060 , $F_{1,25} = 4.74$, $p = 0.039$).

(c) Mesocosm experiment

In two groups, three males sired all genotyped offspring; in the third group, only two males (out of six) sired all offspring (table 1). Most females had mated with more than one male: 19 of 28 broods had two or more fathers (68%; mean number of fathers per brood = 1.8 ± 0.1 across all broods). There was no correlation between the number of genotyped fry in a clutch and the number of fathers assigned ($n = 28$ broods, $r_s = 0.16$, $p = 0.41$), indicating that we did not significantly underestimate the number of sires owing to incomplete genotyping.

To determine which male characteristics influenced mating success, we calculated a generalised linear model with binomial distribution and logit-link function using blackscore, redscore, yellowscore, SL and 'group' as independent variables and the number of matings as dependent variable. The slopes of the relationships between male traits and the number of matings did not differ between the groups (all $F_{1,14} < 0.86$, $p > 0.37$). The minimal adequate model to describe mating success included blackscore only (estimate = 2.19 ± 0.76 , $F_{1,16} = 9.02$, $p = 0.008$; figure 3*a*(i)). Including the effect of redscore did not improve the model ($F_{1,15} = 0.32$, $p = 0.58$). When blackscore was excluded from the model, there was a nearly significant trend for redder males to sire more offspring (estimate = 1.76 ± 0.91 , $F_{1,16} = 4.0$, $p = 0.063$; figure 3*a*(ii)). Redscore and blackscore were strongly correlated ($r_s = 0.76$, $p = 0.0004$).

The unmated males in the experiment had significantly lower blackscores than the mated males, and than the territorial males in our field study (Mann–Whitney U -tests: compared with mated males in the experiment: $n_1 = 10$, $n_2 = 8$, $Z = 2.67$, $p < 0.01$; compared with field males $n_1 = 10$, $n_2 = 28$, $Z = 3.35$, $p < 0.001$; figure 3*b*); whereas the mated males in the experiment did not differ

from the territorial field males ($n_1 = 8$, $n_2 = 28$, $Z = 0.50$, $p = 0.64$). This suggests that the males with low blackscores in our experiments did not become territorial, which in turn implies that most unmated males possessed no territory. This makes the mesocosm data set different from our field and laboratory datasets, which consisted entirely of territorial males. Because mesocosm males were photographed before the experiments, this also suggests that blackscore predicts future territoriality.

We therefore repeated the analysis including only those males that had mated ($n = 8$; seven with high blackscores, one with low). In this subset, redscore emerged as the best, but again non-significant, predictor of mating success (estimate (redscore) = 1.38 ± 0.81 , $F_{1,7} = 3.05$, $p = 0.13$; estimate (blackscore) = 0.61 ± 0.74 , $F_{1,7} = 0.70$, $p = 0.44$). Redscore and blackscore were no longer correlated ($r_s = 0.19$, $p = 0.5$). Thus, this smaller dataset resembled the territorial field males among which redscore and blackscore were also not correlated ($r_s = -0.15$, $p = 0.44$).

4. DISCUSSION

Two different mate-choice experiments and a field study all suggest that the redness of males is the most important criterion for female mate choice among territorial males in *P. nyererei*. In a behavioural laboratory experiment, male redness predicted female response to courtship displays. Probability analysis indicated that the proportion of females preferring the redder one of two males increased with the magnitude of the difference between them. The predicted proportion of redder-preferring females exceeded 50% as soon as there was a difference between the males, suggesting that even a very small difference in redness might be enough to elicit a preference, and associated variation in mating success on population level. However, our experiment included only two male pairs with redscore differences below 10% body coverage. We suspect that we would detect a female response threshold with more trials in this range. Male size and yellowscore did not influence female response; male blackscore significantly decreased female response to male Quiver but did not affect response to Lateral Display or Lead Swim.

The variation in redscores among males in the aquarium experiment resembled that among the field males. In nature, however, females can use cues for mate-choice decisions that were excluded in the experiment, such as chemical signals, territory size and water depth. Our field data reveal that even when these cues are available, male redness is the best predictor of female mate choice, with territory size a secondary factor. Male courtship intensity also predicted female response, possibly because of an interaction between male and female behaviour, with males displaying more as females stay in their territory longer (Collins 1994). In the laboratory experiment, the male enclosures did not allow females to actually follow a male into his spawning pit, making such behavioural interactions less influential. Results of the mesocosm mating experiment under semi-natural conditions are consistent with the field and laboratory results, but apparently highlight the importance of male territoriality for mating success. This may have implications for the potential of intrasexual selection (Seehausen & Schluter 2004; van Doorn *et al.* 2004). The high incidence of multiple paternity that we observed

resembles estimates for Lake Malawi haplochromines (Kellogg *et al.* 1995; Parker & Kornfield 1996; Knight & Turner 2004). In the field we frequently observed males courting females that were already mouthbrooding, illustrating the potential for multiple mating in nature.

Despite the prevalence of multiple paternity, territory ownership appeared to be crucial for male reproductive success. All matings in the mesocosms were monopolized by the blackest males in each group, except for one male with a near-zero blackscore that still mated with four females. Comparison with the blackscores of territorial field males suggests that blackscore is a correlate of territoriality. Even in aquaria, males (as well as females) switch on their black vertical bars when they become territorial. This is generally true in vertically barred haplochromines (Seehausen *et al.* 1999a) and is also observed in other cichlid species (Baerends & Baerends-Van Roon 1950; Barlow & Munsey 1976; Hulscher-Emeis 1992). Males used in the mesocosm experiment were photographed after an acclimatization period of three weeks in the ponds but before the experiments were started. Although group composition in this period was different from that in the experiment, the relationship between blackscore and reproductive success suggests that territoriality during acclimatization predicted territoriality during the experiment. If this suggestion is correct, it implies that alternative mating tactics are not very important in this species, which is consistent with observations in Lake Malawi haplochromines (Parker & Kornfield 1996) and with the theoretical prediction that alternative strategies primarily evolve when paternal investment is high (Taborsky 2001). Male *Pundamilia* do not contribute to brood care, and paternal investment is confined to costs related to territoriality. We also never observed parasitic spawnings by non-territorial 'sneaker' males in the field, but spawning was observed only five times in 195 focal observations.

Unfortunately, the water in the ponds quickly became rather turbid and this made direct observations of male territoriality impossible. At the same time, high algal density hampers light transmission and selectively removes red light. This may provide an additional explanation for the finding that male reproductive success was not significantly related to redscore.

The correlation between redscore and blackscore among mesocosm males disappeared once unmated males were excluded, suggesting that red coloration is not fully expressed until males establish territories, a plausible strategy if the expression of coloration is costly. As a consequence, female choice would have been limited to only two or three males in each mesocosm group. With two or three territorial males in each pond, the territory density resembles the field situation in which territories typically occupy *ca.* 3 m².

We conclude that the conspicuous red coloration of male *P. nyererei*, which is important in behavioural reproductive isolation between this species and its blue sister species (Seehausen & Van Alphen 1998), is subject to directional sexual selection by female mate choice within *P. nyererei*. This is consistent with a speciation scenario in which sexual selection through female choice has played an important role during the divergence of *P. nyererei* from *P. pundamilia*. Although we cannot currently prove it beyond doubt, all evidence suggests that *P. pundamilia* with metallic blue-grey males, and females preferring them over red

males (Seehausen & Van Alphen 1998), represents the ancestral condition (Seehausen *et al.* 1997). *Pundamilia pundamilia* has a wider and more continuous geographical distribution, whereas that of *P. nyererei* is nested within the latter; and blue-grey is the dominant male colour pattern in the genus *Pundamilia* in general (Seehausen & Van Alphen 1999).

Colour polymorphisms in male nuptial coloration, involving blue-red or blue-yellow sister species and intra-specific morphs are common in haplochromine cichlids (Seehausen *et al.* 1999b). We have shown that male nuptial coloration is also under directional sexual selection within a species. Similar results were recently obtained in a cichlid population of Lake Malawi (Pauers *et al.* 2004). The next challenge is to explain why and when such sexual selection becomes disruptive. This requires investigations into proximate (e.g. sensory) and ultimate causes of individual variation in mating preferences (Jennions & Petrie 1997).

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REFERENCES

- Albertson, R. C., Markert, J. A., Danley, P. D. & Kocher, T. D. 1999 Phylogeny of a rapidly evolving clade: the cichlid fishes of Lake Malawi, East Africa. *Proc. Natl Acad. Sci. USA* **96**, 5107–5110.
- 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.
- Arnqvist, G., Edvardsson, M., Friberg, U. & Nilsson, T. 2000 Sexual conflict promotes speciation in insects. *Proc. Natl Acad. Sci. USA* **97**, 10 460–10 464.
- 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.
- Barlow, G. W. & Munsey, J. W. 1976 The red devil-Midas-arrow cichlid species complex in Nicaragua. In *Investigations of the ichthyofauna of Nicaraguan lakes* (ed. T. B. Thorson), pp. 359–369. Lincoln, NB: School of Life Sciences, University of Nebraska.
- Barracough, T. G., Harvey, P. H. & Nee, S. 1995 Sexual selection and taxonomic diversity in passerine birds. *Proc. R. Soc. Lond. B* **259**, 211–215.
- Boake, C. R. B. 2002 Sexual signaling and speciation, a micro-evolutionary perspective. *Genetica* **116**, 205–214.
- Boake, C. R. B., DeAngelis, M. P. & Andreadis, D. K. 1997 Is sexual selection and species recognition a continuum? Mating behavior of the stalk-eyed fly *Drosophila heteroneura*. *Proc. Natl Acad. Sci. USA* **94**, 12 442–12 445.
- Collins, S. 1994 Male displays: cause or effect of female preference? *Anim. Behav.* **48**, 371–375.

- Fryer, G. 1996 Endemism, speciation and adaptive radiation in great lakes. *Environ. Biol. Fish.* **45**, 109–131.
- Gage, M. J. G., Parker, G. A., Nylin, S. & Wiklund, C. 2002 Sexual selection and speciation in mammals, butterflies and spiders. *Proc. R. Soc. Lond. B* **269**, 2309–2316. (doi:10.1098/rspb.2002.2004)
- Gray, D. A. & Cade, W. H. 2000 Sexual selection and speciation in field crickets. *Proc. Natl Acad. Sci. USA* **97**, 14 449–14 454.
- Houde, A. E. & Hankes, M. A. 1997 Evolutionary mismatch of mating preferences and male colour patterns in guppies. *Anim. Behav.* **53**, 343–351.
- Hulscher-Emeis, T. M. 1992 The variable colour patterns of *Tilapia zillii* (Cichlidae): integrating ethology, chromatophore regulation and the physiology of stress. *Neth. J. Zool.* **42**, 525–560.
- Ihaka, R. & Gentleman, R. 1996 R: a language for data analysis and graphics. *J. Comput. Graph. Statist.* **5**, 299–314.
- Irwin, D. E., Bensch, S. & Price, T. D. 2001 Speciation in a ring. *Nature* **409**, 333–337.
- Jennions, M. D. & Petrie, M. 1997 Variation in mate choice and mating preferences: a review of causes and consequences. *Biol. Rev. Camb. Phil. Soc.* **72**, 283–327.
- Johnson, T. C., Scholtz, 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.
- Johnson, T. C., Kelts, K. & Odada, E. 2000 The holocene history of Lake Victoria. *Ambio* **29**, 2–11.
- Kellogg, K. A., Markert, J. A., Stauffer, J. R. & Kocher, T. D. 1995 Microsatellite variation demonstrates multiple paternity in lekking cichlid fishes from Lake Malawi, Africa. *Proc. R. Soc. Lond. B* **260**, 79–84.
- Kirkpatrick, M. & Ravigne, V. 2002 Speciation by natural and sexual selection: models and experiments. *Am. Nat.* **159**, S22–S35.
- 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* **271**, 675–680. (doi:10.1098/rspb.2003.2639)
- Kocher, T. 2004 Adaptive evolution and explosive speciation: the cichlid fish model. *Nature Rev. Genet.* **5**, 288–298.
- Lande, R. 1981 Models of speciation by sexual selection on polygenic traits. *Proc. Natl Acad. Sci. USA* **78**, 3721–3725.
- Masta, S. E. & Maddison, W. P. 2002 Sexual selection driving diversification in jumping spiders. *Proc. Natl Acad. Sci. USA* **99**, 4442–4447.
- 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* **267**, 1049–1061. (doi:10.1098/rspb.2000.1109)
- Parker, A. & Kornfield, I. 1996 Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Environ. Biol. Fish.* **47**, 345–352.
- 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. Lond. B* (Suppl.) Published online 2 July 2004. (doi:10.1098/rspb.2004.0215)
- Schilthuisen, M. 2003 Sexual selection on land snail shell ornamentation: a hypothesis that may explain shell diversity. *BMC Evol. Biol. (Electronic Resource)* **3**, 13.
- Seehausen, O. 1996 *Lake Victoria rock cichlids; taxonomy, ecology and distribution*. Zevenhuizen: Verduijn.
- Seehausen, O. & Bouton, N. 1997 Microdistribution and fluctuations in niche overlap in a rocky shore cichlid community in Lake Victoria. *Ecol. Freshwat. Fish* **6**, 161–173.
- Seehausen, O. & Schluter, D. 2004 Male–male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes. *Proc. R. Soc. Lond. B* **271**, 1345–1353. (doi:10.1098/rspb.2004.2737)
- Seehausen, O. & Van Alphen, J. J. M. 1998 The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). *Behav. Ecol. Sociobiol.* **42**, 1–8.
- 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.
- Seehausen, O., Lippitsch, E., Bouton, N. & Zwennes, H. 1998 Mbipi, the rock-dwelling cichlids of Lake Victoria: description of three new genera and fifteen new species (Teleostei). *Ichthyol. Explor. Freshwat.* **9**, 129–228.
- Seehausen, O., Mayhew, P. J. & Van Alphen, J. J. M. 1999a Evolution of colour patterns in East African cichlid fish. *J. Evol. Biol.* **12**, 514–534.
- Seehausen, O., Van Alphen, J. J. M. & Witte, F. 1999b Can ancient colour polymorphisms explain why some cichlid lineages speciate rapidly under disruptive sexual selection? *Belgian J. Zool.* **129**, 43–60.
- Seehausen, O., Van Alphen, J. J. M. & Witte, F. 1997 Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* **277**, 1808–1811.
- Streelman, J. T., Alfaro, M., Westneat, M. W., Bellwood, D. R. & Karl, S. A. 2002 Evolutionary history of the parrotfishes: biogeography, ecomorphology, and comparative diversity. *Evolution* **56**, 961–971.
- Stuart-Fox, D. & Owens, I. P. F. 2003 Species richness in agamid lizards: chance, body size, sexual selection or ecology? *J. Evol. Biol.* **16**, 659–669.
- Taborsky, M. 2001 The evolution of bourgeois, parasitic, and cooperative reproductive behaviors in fishes. *J. Hered.* **92**, 100–110.
- Taylor, M. I., Meardon, F., Turner, G., Seehausen, O., Mrosso, H. D. J. & Rico, C. 2002 Characterization of tetranucleotide microsatellite loci in a Lake Victorian, haplochromine cichlid fish: a *Pundamilia pundamilia* × *Pundamilia nyererei* hybrid. *Mol. Ecol. Notes* **2**, 443–445.
- Uy, J. A. C. & Borgia, G. 2000 Sexual selection drives rapid divergence in bowerbird display traits. *Evolution* **54**, 273–278.
- van Doorn, G. S., Dieckmann, U. & Weissing, F. J. 2004 Sympatric speciation by sexual selection: a critical re-evaluation. *Am. Nat.* **163**, 709–725.
- 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.
- Venables, W. N. & Ripley, B. D. 2002 *Modern applied statistics with S*. New York: Springer.
- West-Eberhard, M. J. 1983 Sexual selection, social competition, and speciation. *Q. Rev. Biol.* **58**, 155–183.
- Wiernasz, D. C. & Kingsolver, J. G. 1992 Wing melanin pattern mediates species recognition in *Pieris occidentalis*. *Anim. Behav.* **43**, 89–94.
- Witte, Maas, E. & Witte, F. 1985 *Haplochromis nyererei, a new cichlid fish from Lake Victoria named in honour of Mwalimu Julius Nyerere, president of Tanzania*. Leiden: Brill.

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