

Preference polymorphism for coloration but no speciation in a population of Lake Victoria cichlids

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Female mating preference based on male nuptial coloration has been suggested to be an important source of diversifying selection in the radiation of Lake Victoria cichlid fish. Initial variation in female preference is a prerequisite for diversifying selection; however, it is rarely studied in natural populations. In clear water areas of Lake Victoria, the sibling species *Pundamilia pundamilia* with blue males and *Pundamilia nyererei* with red males coexist, intermediate phenotypes are rare, and most females have species-assortative mating preferences. Here, we study a population of *Pundamilia* that inhabits turbid water where male coloration is variable from reddish to blue with most males intermediate. We investigated male phenotype distribution and female mating preferences. Male phenotype was unimodally distributed with a mode on intermediate color in 1 year and more blue-shifted in 2 other years. In mate choice experiments with females of the turbid water population and males from a clearer water population, we found females with a significant and consistent preference for *P. pundamilia* (blue) males, females with such preferences for *P. nyererei* (red) males, and many females without a preference. Hence, female mating preferences in this population could cause disruptive selection on male coloration that is probably constrained by the low signal transduction of the turbid water environment. We suggest that if environmental signal transduction was improved and the preference/color polymorphism was stabilized by negative frequency-dependent selection, divergent sexual selection might separate the 2 morphs into reproductively isolated species resembling the clear water species *P. pundamilia* and *P. nyererei*. *Key words*: cichlids, hybridization, mate choice, polymorphism, sexual selection. [*Behav Ecol* 19:177–183 (2008)]

Variation in female mating preferences within populations can contribute to the maintenance of polymorphism in male secondary sexual characters and is required for speciation by sexual selection. Models for sympatric speciation by sexual selection have assumed either polygenic inheritance of mating preferences with large and symmetrically distributed initial variation (e.g., Schluter and Price 1993; Higashi et al. 1999; Kawata and Yoshimura 2000; Takimoto et al. 2000) or monofactorial inheritance with dominance (Turner and Burrows 1995). However, only a few empirical studies have reported evidence for intrapopulation polymorphism in mating preference for a variable male trait, having the potential to facilitate or drive speciation (Seehausen, van Alphen, and Lande 1999; Brooks and Endler 2001; Morris et al. 2003).

Sexual selection has been suggested as an important force in speciation in the species flock of Lake Victoria haplochromine cichlid fish that consists of more than 500 species (Dominey 1984; Seehausen et al. 1997). The flock emerged within the lake from one or a few ancestral species (Sage et al. 1984; Meyer et al. 1990; Nagl et al. 2000; Seehausen et al. 2003) in possibly less than 16 500 years (Beuning et al. 2002). For many years, biologists have been intrigued by this extremely fast speciation. Cichlids have a flexible and versatile feeding morphology that has allowed rapid specialization for many

different food resources (Liem 1973; Galis and Drucker 1996; Kocher 2004; Albertson and Kocher 2006). However, closely related species usually share very similar morphology, although having very different male nuptial coloration. Several empirical studies have suggested divergence by sexual selection in cichlid fishes from Lake Victoria and Lake Malawi (McKaye et al. 1984; Seehausen et al. 1997; Seehausen and van Alphen 1998; Van Oppen et al. 1998; Seehausen and van Alphen 1999; Seehausen, van Alphen, and Lande 1999; Smith and Kornfield 2002; Knight and Turner 2004; Kocher 2004).

Two sibling species of rock-dwelling haplochromines, *Pundamilia pundamilia* (Seehausen et al. 1998) and *Pundamilia nyererei* (Witte-Maas and Witte 1985), co-occur in many different localities in Lake Victoria (Seehausen 1997). Spawning territories of *P. nyererei* males tend to be in deeper water than those of *P. pundamilia* males, but this varies between islands and depth ranges largely overlap in many areas. The difference in mean territory depth between territories of males of the 2 species decreases with increasing water turbidity (Seehausen 1996). The most apparent difference between the species is male nuptial coloration. *Pundamilia pundamilia* males are blue-gray, and *P. nyererei* males have a bright red dorsum and yellow flanks. Females of both species are cryptically colored and difficult to distinguish. Females are mouth brooders. Mate choice experiments in the laboratory under normal and monochromatic light, which masked color differences, showed that male coloration is an important cue for female choice (Seehausen and van Alphen 1998). Even closely related cichlid fish species of Lake Victoria can exhibit very divergent long wavelength-sensitive LWS opsin genes, and

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Received 23 March 2007; revised 20 October 2007; accepted 21 October 2007.

such divergence can coincide with divergence in male coloration (Carleton et al. 2005). On the other hand, cichlids of this lake do not express ultraviolet (UV)-sensitive opsin genes, probably an adaptation to the low transmission of UV light in eutrophic water (Carleton et al. 2005). The interspecific difference in female mating preference is heritable and most likely oligogenic (Haesler and Seehausen 2005).

Males are less brightly colored in more turbid water, which may indicate that color is costly and tends to get lost when visibility decreases (Reimchen 1989; Seehausen et al. 1997; Maan 2006). In clear water, *P. pundamilia* and *P. nyererei* are reproductively isolated incipient species. Directional sexual selection on male red coloration by *P. nyererei* females was weaker in a turbid water population than in a clear water population (Maan 2006). At the same time, hybridization between the red and blue populations is frequent in turbid water populations (Seehausen et al. 1997). In extremely turbid waters (Secchi disk reading < 70 cm), male coloration is unimodally distributed, with reddish and blue males at the opposite extremes, but most males are of intermediate color, as shown in 2 data sets (Seehausen 1997; Dijkstra et al. 2007). Both data sets suggest a unimodal distribution of male phenotypes, and one set suggests a bias in phenotypes toward blue. The data sets were small ($n = 28$ and $n = 111$ males, respectively). Here, we present a larger data set and time series on male phenotype distribution. Furthermore, to test the critical assumption of models of sympatric speciation by sexual selection, namely, the presence of female preference polymorphism in a single population, we conducted mate choice experiments with females of this turbid water population and males with real blue and real red phenotypes from a clearer water site. We predicted that if sexual selection was a driving force in speciation of these cichlids, we should find between-female variation in their preference for blue versus red males.

MATERIALS AND METHODS

Fish for mate choice experiments

Females were collected at Luanso Island in the southern Mwanza Gulf of Lake Victoria in February 2003 ($n = 11$) and in August/September 2005 ($n = 19$). Females were caught by angling with worm-baited hooks at water depths between 0.5 and 1.5 m between shoreline rocks. To test for female preference polymorphism, we measured each female's response ratio to red and blue stimulus males in mate preference trials. To have really red and really blue males, we collected the males (15 blue *P. pundamilia* and 15 red *P. nyererei*) at Python Islands, 12 km further north in the Mwanza Gulf where the water is clearer. At the same time, we collected 3 *P. pundamilia* and 3 *P. nyererei* females from these islands to test mate preferences. At Python Islands, *P. pundamilia* and *P. nyererei* are largely assortative mating incipient species (Seehausen et al. 1997). *Pundamilia pundamilia* fish were caught by angling and *P. nyererei* fish by angling and gill netting in February 2003. Even though hybrids are occasionally seen, the male color phenotype distribution is distinctly bimodal (Seehausen 1997; Dijkstra et al. 2007). The mean visibility, based on a long time series of Secchi disk readings, is 50 ± 7 cm at Luanso Island and 98 ± 12 cm at Python Islands (Carleton et al. 2005).

Fish were shipped to the Netherlands and kept in aquaria at 24 ± 1 °C and 12:12-h light:dark cycle. The fish were fed a mixture of fresh shrimps and peas 3 days a week and dry commercial cichlid pellets the other days. All fish were individually tagged by inserting a microchip (UKID122GL, Biomark Inc., ID) into the abdominal cavity. Standard length (SL) and body depth were measured with digital calipers (± 0.01 mm), and fish were weighed (± 0.1 g). Gravidity

of females was scored on a 5-point scale (Seehausen and van Alphen 1998), and minimum score for testing was 4. Throughout the experiment, females were kept in individual isolation without any visual contact with males starting 1 week before the onset of the trials. Males were kept in single-species groups.

Mate choice experiments

We used a free-contact 2-way mate choice design in which females chose between one *P. nyererei* and one *P. pundamilia* male from Python Islands. Trials with females collected in February 2003 were conducted between September and November of the same year. Females collected in August/September 2005 were tested between November 2005 and January 2006. An experimental tank of $300 \times 100 \times 60$ cm (length \times width \times height) was divided into 3 compartments with plastic grids with a mesh size of 20×30 mm, which allows the passage of females but restricts the males to their compartment due to their greater body depth. The experimental aquaria were illuminated with high-frequency 58-W Philips fluorescent lamps. In each male compartment, we built a cave of 2 standing bricks with one brick as a roof. The caves were accepted by the males as their territories. Males were placed in these compartments the day before a trial to allow them to settle and become territorial. The test female was introduced into the middle compartment 15 min prior to the start of a trial but was separated from the males by opaque plastic divisions. The latter were removed when the trial started. Each female was tested once with 6 different male pairs. Four male pairs were matched by SL with less than 13% difference. Two additional male pairs were assembled by reusing males from the first 4 pairs in 2 different combinations. This allowed us to test for effects of male species on female preference rather than preference for a specific individual. Three male pairs had the blue male on the left side and the red male on the right side, and in the 3 other pairs, the species were reversed. Two or 3 juvenile tilapias (*Oreochromis niloticus*) were placed in the middle compartment as dither fish to reduce stress of the test female (Haesler and Seehausen 2005). These dither fish did not interfere with courtship behavior. Male courtship behavior and female response were recorded for 20 min with Observer 3 software (Noldus, Wageningen, The Netherlands).

Male courtship behavior usually starts with an approach of the male, after which the male shows his side and starts to shake his body, the so-called quiver (illustrated in Seehausen 1996). If the female responds positively by approaching the male, the latter will try to lead the female to the nest and starts circling. In these trials, spawning did not take place because the trials were stopped before spawning ensued. Successful trials used for data analysis were those in which both males courted at least once and females responded positively to one or both males. In total, we conducted 241 trials to obtain 66 successful trials with 11 females collected in 2003 and 114 successful trials with 19 females collected in 2005.

Data analysis female response ratio

Individual female response ratio as well as female response ratio at population level were estimated with a generalized linear model (GLM) with binomial distribution with logit link function in R software (version 2.4.0, Ihaka and Gentleman 1996). Response ratio is defined as the ratio of the number of positive responses of the female to the total number of courtship displays of the male. Variables in the model were male species and position of the males in the tank (left or right). Furthermore, we included SL, body depth, weight, or condition factor of the males (Maan et al. 2004). Condition factor

of the males was calculated as the residuals of a log-log regression of weight on SL (Reist 1985; Meka and McCormick 2005). Condition factor was also calculated by $100 \times \text{weight (gram)} / \text{SL (centimeter)}^{2.76}$ (Bolger and Connolly 1989; Craig et al. 2005). We fitted models, which included each trial as a fixed effect, to correct for differences in courtship frequency of the males in 1 trial. Male species and female identity and the interaction between the 2 covariates were included in the model to obtain estimates of preference of individual females. A positive response ratio indicates a net preference for *P. nyererei*, whereas a negative response ratio is a net preference for *P. pundamilia*. We checked models for overdispersion and adjusted statistics by switching to *F*-statistics and a quasi-likelihood approach when there was significant overdispersion. Maximum models were simplified stepwise using chi-square tests, and covariates with nonsignificant tests were removed from the models. To test for differences in preference of females collected in 2003 and 2005, a separate model was built in which year was included. To test whether there were differences in courtship behavior between males of the 2 species from the clearer water island, we fitted a GLM with Poisson distribution to the data. We took the number of courtship behaviors performed by the males as the response variable and male identity and male species as covariates.

Male phenotype distribution

Males were collected at Luanso Island by angling in April and August 2001, February 2003, and January and May 2005. Photos were taken of, in total, 241 individuals. The photos were scored on a 0- to 4-color scale by 5 different observers (we adjusted the 0- to 5-color scale of Dijkstra et al. [2007] by merging categories 2 and 3 into category 2; we renumbered former category 4 into 3 and 5 into 4). Blue is scored as 0; 1 is a yellow flank but no red, spiny part of dorsal fin is blue; 2 is yellow flank with some red on the flank along the upper lateral line, spiny dorsal fin is blue; 3 is yellow flank with a partially red dorsum upward from the upper lateral line, but a gray body crest and largely blue spiny dorsal fin; and 4 is yellow flank with a completely red dorsum between the upper lateral line and the body crest, red spiny dorsal fin (Figure 1). The correlation between the phenotype scoring of the different observers was calculated in SPSS12.0.1 (SPSS Inc., Chicago, IL). Further data analysis was done by fitting multinomial log-linear models with the multinom package in R software (Venables and Ripley 2002). The models were compared with likelihood ratio (LR) tests.

RESULTS

Female response ratio estimation with GLMs

Individual female response ratio was not affected by male SL, male body depth, male weight, male condition factor (using either of the 2 methods), male position in the tank, or female year of collection. Eliminating these variables from the maximum model did not significantly affect the predictions of the model compared with the minimal adequate model. The minimal adequate model describing female response ratio included trial number ($F_{180, 180} = 1.313$, $P = 0.034$), interaction between female identity and male species ($F_{35, 180} = 3.259$, $P < 0.001$), female identity, and male species (*F*-test results are not available because elimination of these terms is not possible when the interaction term is in the model). At $\alpha = 0.05$, 2 females had a significant preference for *P. nyererei* males (Figure 2, gray bars), whereas 7 females showed a significant preference for *P. pundamilia* males (Figure 2, dashed bars). Three females showed a trend ($0.05 < P < 0.10$) for preferring

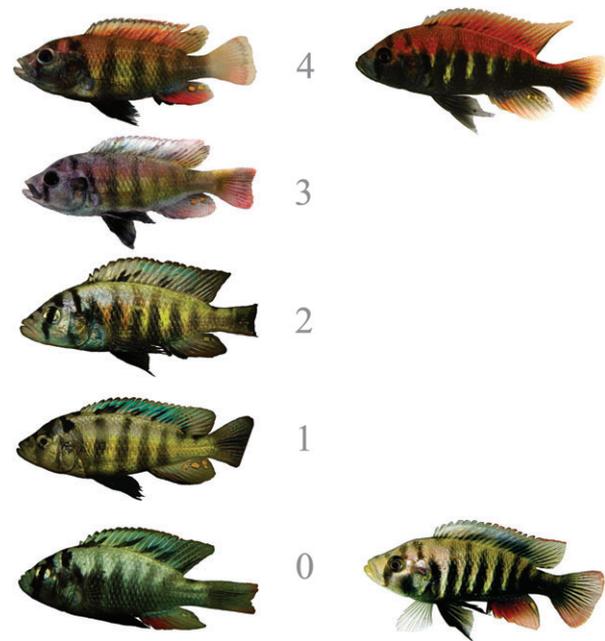


Figure 1

Male phenotype color scale. Luanso males are on the left side, and for comparison, *Pundamilia nyererei* (top) and *Pundamilia pundamilia* (bottom) males of Python Islands on the right.

P. pundamilia males. The remaining 18 females did not show any preference between males of the blue and the red species ($P > 0.10$, Figure 2, open bars). All 6 *P. nyererei* and *P. pundamilia* females of Python Islands showed significant preferences for males of their own species (black bars labeled with “N” and “P,” respectively, in Figure 2).

Female response ratio at population level was affected by trial number ($F_{179, 179} = 1.378$, $P = 0.016$) and by male species ($F_{1, 179} = 8.085$, $P = 0.005$). Overall, there was no significant preference for one of the male species. There was no difference in courtship frequency between males of the 2 species ($F_{1, 420} = 0.528$, $P = 0.468$).

Male phenotype distribution

Phenotype scoring of different observers was very similar and significantly positively correlated (Spearman rank correlations between 0.605 and 0.729, $P < 0.05$). To test for differences between years with multinomial models, we took the mean score of all observers per male. The model that included year as a fixed effect was significantly different from the model without year and only the intercept (degrees of freedom [df] = 8, LR = 19.6, $P = 0.013$). Male phenotype distribution differed significantly between 2001 and 2003 (df = 4, LR = 11.2, $P = 0.024$) and between 2003 and 2005 (df = 4, LR = 10.9, $P = 0.031$) but not between 2001 and 2005 (df = 4, LR = 8.90, $P = 0.064$). The predictions of the multinomial models were plotted per year (Figure 3). Male phenotype was unimodally distributed in all years, intermediate in 2003 and blue-shifted (to the left) in 2001 and 2005.

DISCUSSION

Young species flocks, like the cichlid fish of Lake Victoria, provide unique opportunities to study speciation and coexistence of nascent species. Divergent female mating preferences are important in maintaining phenotypic distinctiveness of

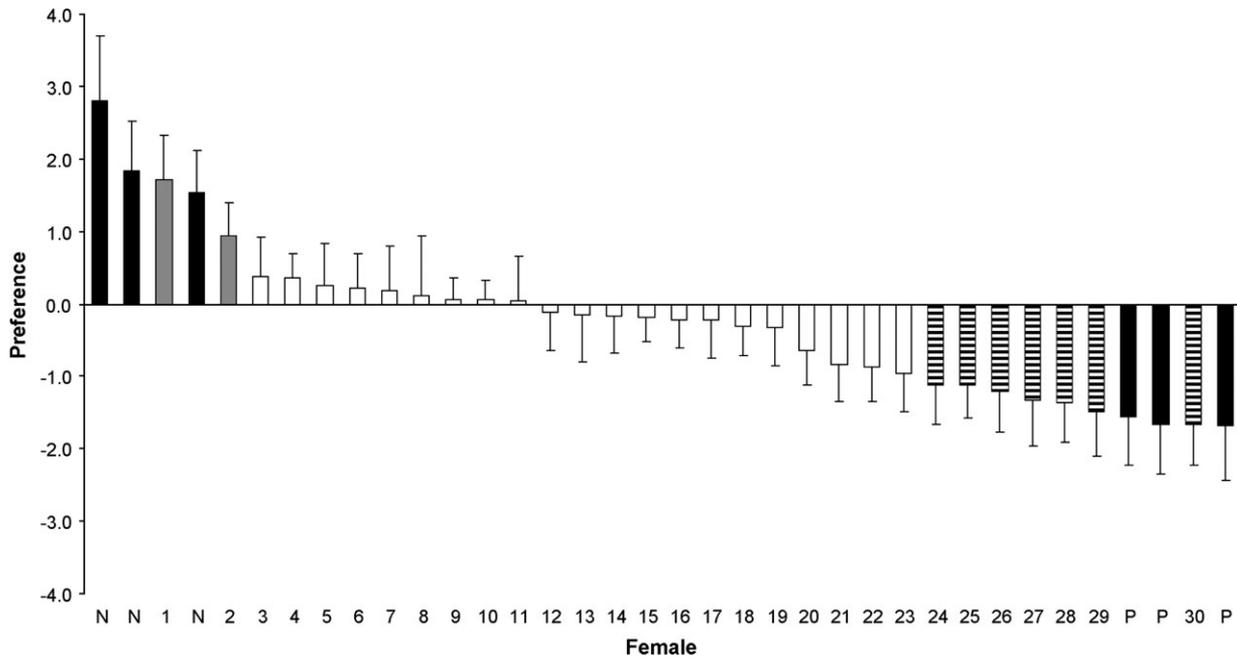


Figure 2 Mate preference of females of a turbid water population for *Pundamilia nyererei* males (positive score on y axis) or *Pundamilia pundamilia* males (negative score on y axis) with 1 standard error. Female preference is expressed as a net response ratio for red and was based on a GLM. Gray bars represent females with a significant preference for *P. nyererei* males, and dashed bars represent females with a significant preference for *P. pundamilia* males. Females without a preference for one of the male species are shown by open bars. Black bars labeled with an “N” represent *P. nyererei* females and black bars labeled with a “P” are *P. pundamilia* females from Python Islands, which all showed significant preference for males of their own species. Each female was tested 6 times.

closely related and sympatric species of cichlids, and they may have been important in the process of speciation as well (Seehausen et al. 1997; Seehausen and van Alphen 1998; Van Oppen et al. 1998; Seehausen 2000; Knight and Turner 2004). Disruptive selection by female mating preferences can theoretically facilitate sympatric speciation in specific situations (Turner and Burrows 1995; Payne and Krakauer 1997; Higashi et al. 1999; Takimoto et al. 2000) and, perhaps, also in more broadly applicable situations when interacting with some form of negative frequency-dependent selection (van Doorn et al. 2004). For female mate choice to exert disruptive selection on male traits, the distribution of female preference variation has to be broader than the distribution of male phenotypes. Apart from one study that used laboratory-reared

females (Seehausen, van Alphen, and Lande 1999), this had never been tested to date in any cichlid fish population and never with wild-caught females.

Here, we have investigated a population of the species-rich genus *Pundamilia* from Lake Victoria. In most sites with clear water, several fully sympatric species of *Pundamilia* coexist (Seehausen and van Alphen 1999). Such coexisting species usually differ in male breeding coloration (Seehausen and van Alphen 1999; Seehausen and Schluter 2004), the main phenotypes being blue, red dorsum, and red chest (Seehausen, van Alphen, and Witte 1999). The population that we studied here inhabits extremely turbid water (Luanso Island). The frequency distribution of male color phenotypes suggests a single panmictic population with variation in male dorsum

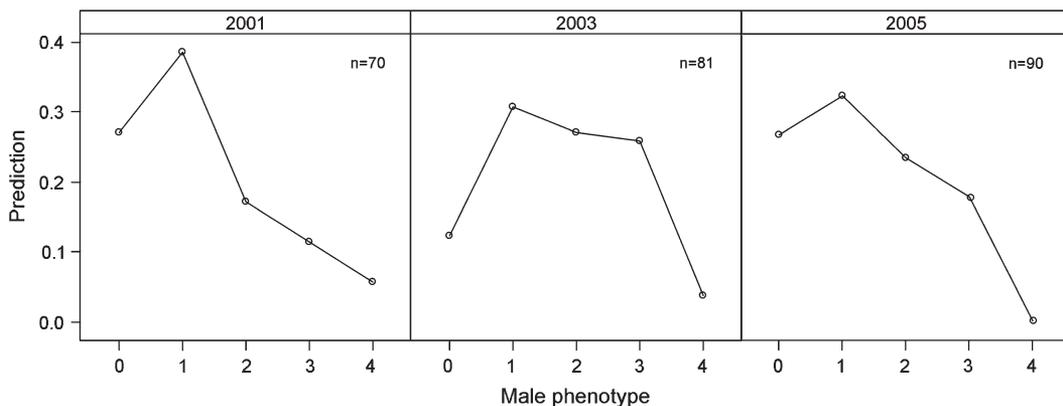


Figure 3 Male phenotype distribution of a turbid water population. Color categories on the x axis ranges from blue (0) to red (4). The predictions of the frequency of males calculated by the multinomial model are on the y axis. Every plot represents a different year and *n* is the number of males in the data set.

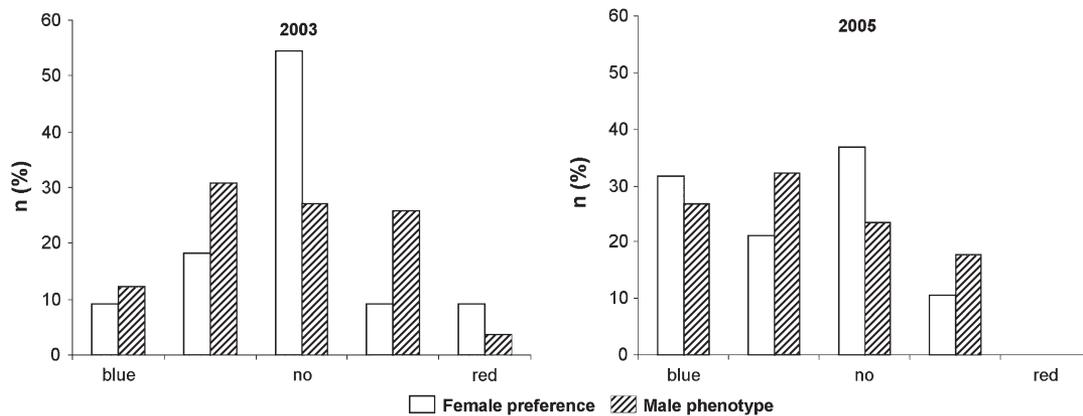


Figure 4

Female preference and male phenotype distribution for 2003 (left panel) and 2005 (right panel). On the *x* axis are the 5 categories, and on the *y* axis the number of individuals as a percentage of the total number of individuals are shown.

coloration (in agreement with Seehausen 1997; Dijkstra et al. 2007). We tested 30 wild-caught females from this population for variation in mate preferences between males of 2 closely related species from the nearest location with clearer water, one with blue males (*P. pundamilia*) and one with red dorsum males (*P. nyererei*).

We found significant between-female variation in mating preferences for red and blue males in the turbid water population. Two females (7%) showed a significant preference for males of the red species, 7 (23%) for males of the blue species, and the remaining 21 females (70%) did not show any preference, even though 3 showed a trend toward preferring blue males. The preferences at the extremes of the distribution are well within the range of species-specific preferences at the clearer water location. If preference was determined by many loci, we would expect that preference was lost in a population that mates at random. That 30% of the females in the turbid water population had significant preferences is consistent with the conclusion from a behavioral genetics study that preference is determined by few loci (Haesler and Seehausen 2005). However, it could alternatively be explained by some deviation from random mating retaining multiple preference loci in weak linkage disequilibrium.

Females of the red and blue species from clear water populations differed in their visual sensitivity, which may coincide with their preference for male nuptial coloration (Carleton et al. 2005; Maan et al. 2006). In contrast, Carleton et al. (2005) found no variation in the LWS pigments in the Luanso population. The sample size was small, but a much larger new data set confirms this (Seehausen O, Terai Y, Magalhaes I, Carleton KL, Mrosso HDJ, van der Sluijs I, Schneider MV, Tachida H, Shichida Y, Okada N, unpublished). Hence, we observe standing variation in female preference in the Luanso population despite absence of obvious variation in the visual system.

We do not know whether the Luanso population has always been a single admixed population or whether, alternatively, reproductive isolation has recently broken down between previously differentiated blue and red *Pundamilia* species after the increase in water turbidity that Lake Victoria experienced in the past decades (Verschuren et al. 2002). Even though Lake Victoria was much clearer in the past, historical data also reveal that the interior sections of embayments, such as the southern Mwanza Gulf, were turbid before the recent eutrophication (Graham 1929). It is hence very likely that populations, such as the one we studied here, have existed prior to the recent changes in the system. Whatever the historical

origin of the genetic variation in male color and female preference in *Pundamilia* from Luanso Island might be, our results suggest that such variation can be maintained in a population for at least 12 years, corresponding to about 12 nonoverlapping generations (Seehausen 1997).

The existence of variation in female mating preferences in a single population implies potential for disruptive sexual selection to facilitate or drive speciation (or respeciation). Interestingly, female mating preference distribution closely matched the distribution of male phenotypes and its variation between years (Figure 4). Dijkstra et al. (2007) showed that although most males at Luanso Island are intermediate in color, there is a population-level bias toward blue coloration. Even though 2 of our data sets (2001, 2005) revealed a blue bias too, the 2003 data set did not. However, it would predict that in the absence of another fitness advantage for red males, or negative frequency-dependent selection, the red-colored male phenotype and the red-preferring female phenotype go extinct. Red males may indeed have an advantage over blue males in male-male competition when they are rare. Males in the Luanso population are more aggressive to the more abundant blue phenotype than to the less abundant red phenotype (Dijkstra et al. 2007), and red males dominate blue males in dyadic contests (Dijkstra et al. 2005).

We described a population of cichlid fish that contains variation in male nuptial coloration and variation in female mating preferences for the different nuptial coloration variants. Although, female preference for intermediate male phenotype has not been tested and the intermediate preference categories in Figure 4 refer to females with weak or no preferences between red and blue, the distribution of the variation in male color and female color preferences are remarkably similar. This suggests that variation in one may strongly impact and possibly maintain variation in the other. Models of trait evolution under disruptive sexual selection predict this situation (van Doorn et al. 2004). Our time series is too short to know whether the Luanso population of *Pundamilia* represents a stable or a transient polymorphism. However, the population has been polymorphic for male coloration at least from 1993 (Seehausen 1997; Seehausen et al. 1997) to 2005. By crossing in the laboratory the blue and the red species from the more clear water Python Islands, we have been able to reconstruct a range of male color (Seehausen, forthcoming) and female mating preference (Haesler and Seehausen 2005) phenotypes that very closely match those observed in the wild at Luanso Island. Therefore, we hypothesize with some confidence that the color and preference

genes that segregate in the Luanso population involve the same genes that differentiate the sibling species *P. pundamilia* and *P. nyererei* at other sites in Lake Victoria.

Comparative distribution data (Seehausen et al. 1997) and experimental data (Seehausen and van Alphen 1998; Dijkstra et al. 2006) strongly suggest that the turbid water at Luanso Island constrains evolutionary response to disruptive selection on color and preference. Besides difficulties seeing nuptial colors in turbid water, turbidity also affects encounter rates between males and females, and both effects lead to reduced mate selectivity by females. Loss of female selectivity with decreasing water transparency has also been reported in *Pomatoschistus microps* (common gobies, Reynolds and Jones 1999) and *Pomatoschistus minutus* (sand gobies, Järvenpää and Lindström 2004). Recently, Kronforst et al. (2006) described a polymorphic population of *Heliconius* butterflies, in which random mating between 2 distinct morphs occurred for many generations. They found that males of one morph did not have any mating preference, whereas males from the other morph maintained a significant morph-assortative preference. Thus, random mating does not necessarily lead to the complete and symmetric loss of preference. In our case, we found females with preferences at both sides of the male trait spectrum, suggesting either oligofactorial female preferences or partial assortative mating.

In summary, we found variation in female mate preference in a population of Lake Victoria cichlids, which coincides with a polymorphism in male coloration in the same population. If the clarity of the water would increase and the light spectrum becomes wider, such that different male colors could be advantageous in shallow and deep water, and if preference/color polymorphism was stabilized by negative frequency-dependent selection, divergent sexual selection on male nuptial color might become strong enough to facilitate speciation.

FUNDING

Netherlands Science Foundation (NWO-ALW 810.64.011) and research grants from Leiden University Fund and Schure-Beijerinck-Popping Foundation.

We thank the Tanzanian Fisheries Research Institute for hosting and use of the facilities (Professor Philip Bwathondi, Egid Katunzi) and the Tanzania Commission for Science and Technology for the research permits. Mhoja Kayeba and Mohamed Haluna provided assistance in the field. Furthermore, we thank Kees Hofker for assistance in the laboratory and the field, Tom Van Dooren for his help with the statistics, Frans Witte for interesting discussions, and Martine Maan for her comments on earlier drafts of the manuscript. Peter Dijkstra, Nellie Konijnendijk, and John Mrosso are acknowledged for providing photos of males from Luanso Island. Special thanks to Riet van Dinter, Alan Hudson, Isabel Magalhaes, and Rike Stelkens for male phenotype scoring. The experiments were approved by the Animal Ethics Screening Committee (UDEC 03061).

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