

No evidence for a genetic association between female mating preference and male secondary sexual trait in a Lake Victoria cichlid fish

Inke van der SLUIJS^{1*}, Ole SEEHAUSEN^{2,3}, Tom J. M. Van DOOREN¹, Jacques J. M. van ALPHEN¹

¹ Department of Animal Ecology, Institute of Biology, Leiden University, PO Box 9516, 2300 RA Leiden, The Netherlands

² Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, CH-3012 Bern, Switzerland

³ Department of Fish Ecology & Evolution, Centre of Ecology, Eawag Swiss Federal Institute for Aquatic Science and Technology, Evolution & Biogeochemistry, Seestrasse 97, 6047 Kastanienbaum, Switzerland

Abstract Sexual selection by female mating preference for male nuptial coloration has been suggested as a driving force in the rapid speciation of Lake Victoria cichlid fish. This process could have been facilitated or accelerated by genetic associations between female preference loci and male coloration loci. Preferences, as well as coloration, are heritable traits and are probably determined by more than one gene. However, little is known about potential genetic associations between these traits. In turbid water, we found a population that is variable in male nuptial coloration from blue to yellow to red. Males at the extreme ends of the phenotype distribution resemble a reproductively isolated species pair in clear water that has diverged into one species with blue-grey males and one species with bright red males. Females of the turbid water population vary in mating preference coinciding with the male phenotype distribution. For the current study, these females were mated to blue males. We measured the coloration of the sires and male offspring. Parents-offspring regression showed that the sires did not affect male offspring coloration, which confirms earlier findings that the blue species breeds true. In contrast, male offspring coloration was determined by the identity of the dams, which suggests that there is heritable variation in male color genes between females. However, we found that mating preferences of the dams were not correlated with male offspring coloration. Thus, there is no evidence for strong genetic linkage between mating preference and the preferred trait in this population [*Current Zoology* 56 (1): 57–64 2010].

Key words Speciation, Sexual selection, Mate choice, Genetic association, Cichlid fish, Lake Victoria

Sexual selection plays an important role in the process of speciation (Darwin, 1871; Andersson, 1994). Mating preferences, usually of females, can evolve through direct or indirect benefits. Potential direct benefits to females include access to resources (Saetre et al., 1995; Moller and Jennions, 2001), whereas indirect benefits could entail “good genes” selection for offspring with high fitness (Zahavi, 1975) or Fishers’ runaway selection for sexually attractive offspring (Fisher, 1930; Lande, 1981). Theoretically, through selective mating, genes affecting male secondary sexual traits can become associated with genes affecting female mating preferences for the male trait (Lande, 1981; Kirkpatrick, 1982; Wu, 1985; Turner and Burrows, 1995; Payne and Krakauer, 1997; Higashi et al., 1999; van Doorn et al., 2004; Gavrillets et al., 2007). This process requires that

mating preference and the preferred trait both have a heritable basis. Covariance between mating preference and the preferred trait can also be caused by pleiotropic effects of one gene or tight linkage between genes affecting the traits (Lande, 1981; Kronforst et al., 2006). Genetic associations between the traits can facilitate speciation because, once evolved, covariance protects against recombination due to random mating (Lande, 1981; Kronforst et al., 2006). Empirical evidence for population divergence facilitated by a genetic association between mating preference and a secondary sexual trait, as predicted by models, is scarce. One recent example is the genetic linkage between acoustic preference and song QTL in the rapidly speciating Hawaiian cricket genus *Laupala* (Shaw and Lesnick, 2009). Another example is mating preference in two completely

Received Nov. 09, 2009; accepted Dec. 09, 2009

* Corresponding author. E-mail: inke.vandersluijs@mcgill.ca. Current address: Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montréal, H3A 1B1 Québec, Canada

© 2010 *Current Zoology*

interfertile species of *Heliconius* butterflies, which is genetically linked to the preferred trait (Kronforst et al., 2006). This genetic architecture, in combination with divergent natural selection, may have led to the divergence of two *Heliconius* species within the last 500,000 years (Kronforst et al., 2006). Theoretical models and only a few empirical studies have shown that genetic associations between mating preference and the sexual trait can facilitate speciation.

The speciation of haplochromine cichlid fish in African lakes has been suggested to be driven by interactions of ecological and sexual selection (Seehausen et al., 1997; Kornfield and Smith, 2000; Kocher, 2004; Seehausen, 2006) but in some lakes took place in a much shorter time span. Previous studies have suggested that sexual selection by female mating preference for male coloration played a role in the rapid speciation of haplochromines in Lakes Victoria and Malawi (Dominey, 1984; Seehausen et al., 1997; Seehausen and van Alphen, 1998; Van Oppen et al., 1998; Knight and Turner, 2004; Maan et al., 2004). Males of closely related species differ to a great extent in nuptial coloration (Seehausen, 1996; Genner and Turner, 2005). For several species, females are known to mate positively species-assortative, and their choice is often based on male nuptial coloration (Seehausen and van Alphen, 1998; Knight and Turner, 2004; Stelkens et al., 2008). Directional sexual selection by females on male nuptial coloration has been shown in two species of African cichlids, one from Lake Victoria (Maan et al., 2004) and one from Lake Malawi (Pauers et al., 2004). All haplochromines from these lakes are maternal mouthbrooders. As such, females do not gain direct benefits by their choosiness, since males of Lakes Victoria and Malawi provide neither brood care nor ecological resources. As shown by two different studies, indirect benefits may be obtained through inheritance of good genes or of attractive sons (Taylor et al., 1998; Maan et al., 2006).

Little is known about the genetic architecture of female mating preference and male nuptial coloration of haplochromines. In contrast with *Heliconius* butterfly studies (Kronforst et al., 2006), direct tests for physical linkage between cichlid female mating preferences and male secondary sexual traits are complicated by sex-limited expression of the secondary sexual traits. Genetic linkage between female preferences and male coloration could potentially contribute to rapid speciation in cichlids. In the current study we test for such a genetic association in an admixed but phenotypically variable population of the rapidly radiated genus *Pun-*

damilia. Wild-caught females with a known mate preference were mated to a male of known coloration, and the color of the male offspring was quantified. We expected the male offspring coloration to match the preferred color of the dam if a genetic association existed between preference and the preferred trait.

1 Materials and Methods

1.1 Study species

Cichlid species of the genus *Pundamilia* inhabit rocky shores and islands throughout Lake Victoria (Seehausen, 1996). Seehausen (2009) described the occurrence of two sibling species of this genus in the southern part of the lake along a transect of decreasing water transparency. Luanso Island is at the southern end of the transect in the Mwanza gulf, where the water is very turbid. Around this island, rocky shores are inhabited by a single phenotypically variable *Pundamilia* population in which male nuptial coloration ranges from blue to bright yellow to red, but most males have an intermediate phenotype (Seehausen, 1997; van der Sluijs et al., 2008a). Female mating preferences vary in this population (van der Sluijs et al., 2008a). Many females do not have any preference, but some have significant preferences for blue males, and few have significant preferences for red males. In contrast, the water is relatively clear at Makobe Island at the northern end of the transect in the open lake, where two distinct sister species coexist and no intermediate phenotypes are present (Seehausen, 1997; Dijkstra et al., 2007). Here males of one species, *Pundamilia pundamilia* (Seehausen et al., 1998), are blue, and males of the other one, *P. nyererei* (Witte-Maas and Witte, 1985), have a bright red dorsum and yellow flanks. Females of both species are cryptically colored and difficult to distinguish. Further, the two species show significant neutral genetic and ecological differentiation (Seehausen, 2009). Females of Makobe Island exert vision-mediated preferences for males of their own species (Seehausen, 1997). At the intermediate water transparency of the Python Islands in the Mwanza Gulf, species are strongly differentiated phenotypically but only weakly differentiated genetically (Seehausen, 2009). Females also prefer to mate positively species-assortative at this location (Seehausen and van Alphen, 1998; Seehausen et al., 2008).

Female mating preferences are heritable and probably determined by few genes (Haesler and Seehausen, 2005). Interspecific differences in male nuptial coloration are also heritable (Seehausen, 1997; Seehausen et

al., 1997; van der Sluijs et al., 2008b). F1 hybrid males between *P. pundamilia* and *P. nyererei* are intermediate in coloration, and F2 hybrids segregate for color (van der Sluijs et al., 2008b; Seehausen, 2009). This suggests that male coloration is not determined by a single gene, but not by very many either. These heterospecific crosses yielded fully viable and fertile hybrid offspring (van der Sluijs et al., 2008c). To test whether speciation in this system was aided by genetic linkage between female mating preference and male nuptial coloration, we used females of the naturally variable population at Luanso Island, the turbid water location.

1.2 Mating preferences and fish breeding

Fish were collected from Luanso Island and Python Islands in February 2003 and August/September 2005 and shipped to the University of Leiden, the Netherlands. Female mating preferences at Luanso Island have previously been reported (van der Sluijs et al., 2008a). Mating preferences was determined for 30 females in free-contact two-way choice trials in which females could choose between males of the blue and males of the red species from nearby Python Islands. Each female was tested six times with different male pairs. Response ratios were scored and female preference estimated using generalized linear models (van der Sluijs et al., 2008a).

After determining their mating preferences, all females were mated to blue males *P. pundamilia* from Python Islands. Only 12 of 30 females produced clutches, some of them more than one. In total, 20 clutches of 12 different females were sired by seven different males. Seven females had one clutch, three females had two clutches, one female had three clutches, and one female had four clutches. When females had more than one clutch, these were always sired by different males. Two males sired only one clutch each, three males sired two clutches, one sired four clutches, and one male sired eight clutches from different females. Hence, none of the broods had the same combination of parents. The estimated preferences of the 12 females are shown in Fig. 1.

We reared the male offspring and measured the coloration. We used sires of one species only to standardize the effect of sire on the coloration of the offspring. We chose the blue species as sire because previous study suggested that blue coloration was recessive to red (Seehausen, *personal observations*).

1.3 Photography

Male offspring were photographed after reaching sexual maturity and full adult size at the age of 1 to 1.5

years. Four tanks of $100 \times 20 \times 20 \text{ cm}^3$ (L \times W \times H) each were divided into five compartments by transparent Plexiglas dividers, perforated for water exchange. Males were placed in the compartments one week before the photographs were taken to allow them to become territorial and express nuptial coloration. A Kodak Color strip (No. Q13, Eastman Kodak, Rochester, NY) was attached to the front glass to calibrate the photos in Photoshop 6.0 (Adobe Systems Inc.). In total, 187 male offspring and seven sires were photographed with a Sony digital camera (DSC-F707).

1.4 Color analysis

Color was measured from the calibrated digital photos of all males. Three different color measurements were taken, two quantitative and one categorical. Yellow- and red-colored pixels and the total body area in pixels, excluding fins and eyes, were measured with SigmaScan Pro 4.0 (Systat Inc.). The hue windows were 0 – 26 plus 232 – 255 for red and 27 – 45 for yellow, and the saturation window was 40% – 97% (Maan et al., 2004). Red and yellow coloration were expressed as the percentage red and yellow pixels of the total body area.

For the categorical measurement, males were assigned visually to one of five different color classes, based on their total body coloration (van der Sluijs et al., 2008a), hereafter referred to as color score (Fig. 2). Color score was used because it was not possible to measure the amount of blue coloration quantitatively, and males were assigned to a certain category based on the amount and the location of the color. A male was assigned to category 0 when it was completely blue/grey

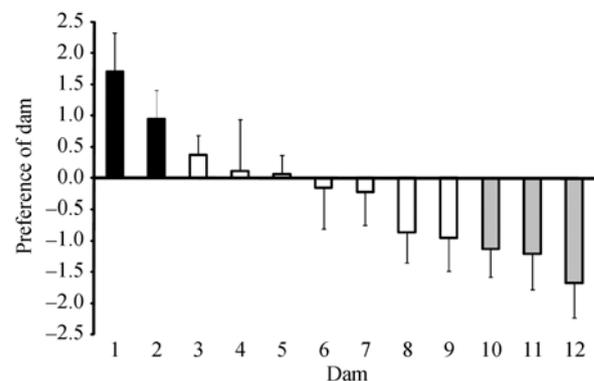


Fig. 1 Mating preferences of 12 females of a population with variable male coloration

A positive score is a preference for males of the red species (black bars), and a negative score is a preference for males of the blue species (grey bars). The open bars are females without a preference, not significantly different from zero. The females are ranked along the x-axis from red-preferring on the left to blue-preferring on the right (modified from van der Sluijs et al., 2008a).

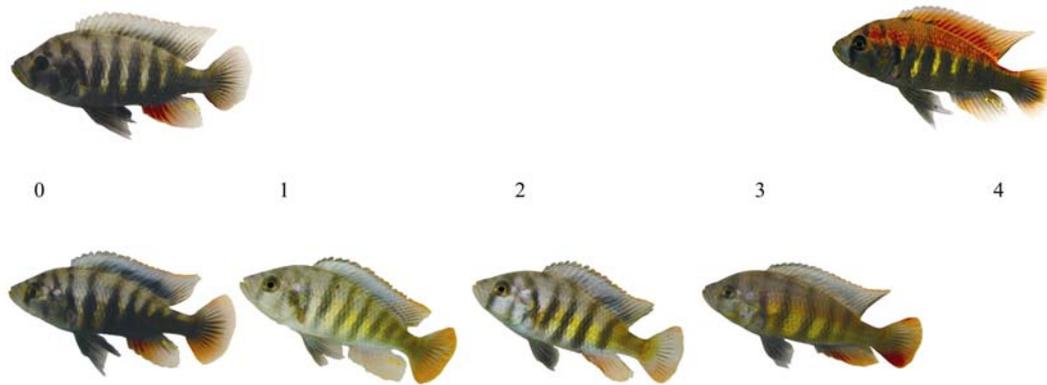


Fig. 2 Male phenotype color scale ranging from blue (score 0) to red (score 4)

Pundamilia pundamilia (top left) males sired the clutches. F1 male phenotypes are shown on the bottom row. None of the male offspring were as red as *P. nyererei* males; therefore, we show a male originating from a clearer water population (top-right, modified from van der Sluijs et al., 2008a)

without any yellow or red on the body. When a male had yellow on the flanks but no red, it was assigned to category 1. When there was additionally some red present on the flanks along the upper lateral line, this was classified as category 2. A male was in category 3 when it had yellow flanks with some red on the dorsum and in category 4 when it had yellow flanks and a completely red dorsum.

1.5 Data analysis

We tested whether the three color measurements were correlated within the individual male offspring with Spearman rank correlations (SPSS 15.0, Inc., Chicago, IL).

The general idea we used to structure our parents-offspring regression is that the correlation between the genotypic value of one trait and the phenotypic value of another trait is equal to the genetic correlation. For traits determined in a polygenic manner and without sex specificity, the expected genotypic value of an offspring is half that of the dam plus half the genotypic value of the sire (Lynch and Walsh, 1997). Since male nuptial coloration is not expressed in females, we can estimate genotypic values of dams only from the phenotypic data of their offspring. On the other hand, phenotypic data for both sires and male offspring can be used to estimate or predict genotypic values of sires.

To determine the genotypic effects of dam and sire on coloration of male offspring, we fitted linear mixed-effect models in R software (version 2.4.0, Ihaka and Gentleman, 1996) and used the lme4 library (version 0.9975-10, Bates and Sarkar, 2006) in order to fit the mixed models. Male coloration traits were the dependent variables, and dam and sire were the grouping variables stratifying the random effects. We investigated separate models for each of the following traits:

percentage yellow, percentage red and color score. Per trait, we combined data on parents and offspring in a single model, such that we used the maximum available data to estimate genotypic variances and predict or estimate genotypic values. In order to discount the genotypic value of a parent in the offspring correctly, random regressions were fit without intercept and with a genotypic weight covariate. For example, in order to estimate the genotypic variance between sires in a mixed model, the weight covariate of the random regression was equal to 1.0 for observations on a sire and 0.5 in the offspring. By stratifying the random regression over sires, we could then estimate the genotypic variance between sires based on data from two generations. Using this approach, estimates of the variances of the random regression slopes of dam and sire are equal to genetic variances, and the heritability of each trait is equal to the variance of the random effect divided by the total phenotypic variance.

For the estimation of random color variation between dams, only data on offspring could be used, and all received a weight of 0.5. When fitting fixed effect models, we fitted models with pairwise interactions between the weight covariate and dam and sire effects. The maximum model contained dam and sire as groups and separate regressions for each effect.

Since there is no software available that estimates the genetic correlation between a normally distributed trait (male coloration) and a binomial trait (mating preference), and in which several random effects can contribute to phenotypic variation at the same time, we tested the genetic correlation separately from fitting genetic and phenotypic variances. Female mating preference was initially estimated with a binomial generalized linear model as described in van der Sluijs et al. (2008a).

We then determined whether the mating preference of the dams was linked to the coloration of their male offspring, by calculating Spearman rank correlations between the estimated preferences of the dams and the predicted dam genotypic value for coloration of the male offspring, based on the minimum adequate mixed-effect model. Spearman rank correlations were used because the female preference parameters used for preference phenotypes were not normally distributed.

We observed significant reduction of the mean in the predicted random effects for dams with small numbers of offspring. Since this substantially affected the ranking of color traits, we also fitted linear models with least-squares fixed effects instead of random effects. Linkage between the estimated mating preference of the dams and the estimates of the fixed effects of the coloration of the male offspring was also tested by Spearman rank correlation tests.

If no significant effect of the sire was found on the coloration of the offspring, we used bivariate mixed-effect models using the SAS NLMIXED Procedure (Gueorguieva, 2001), assuming conditional independence between the two traits (female mating preference and male offspring coloration), given the random variation among offspring from different dams. That allowed us to estimate the genetic correlation between female mating preference and male offspring coloration along with the other parameters in a single fit. We used adaptive gaussian quadrature (AGQ) with ten quadrature points to approximate the likelihood of a model. Since we could only fit a single random grouping level in this approach, there were no corrections made for variation in response among different mate choice trials.

Conservative likelihood-ratio tests or F tests were used to assess the significance of random effects and to simplify the model if warranted. From the minimum adequate models selected, random effects were extracted or parameter estimates inspected. The bivariate models we fitted using SAS only included data on the offspring,

since variation between sires was not considered there.

2 Results

2.1 Individual color measurements

Within individuals, the percentage of red coloration was significantly positively correlated with the percentage of yellow coloration (Spearman $r_s = 0.521$, $P < 0.001$, $n = 187$). Further, the percentage of red coloration was also positively correlated with the 0–4 color score (Spearman $r_s = 0.261$, $P < 0.001$, $n = 187$). In contrast, the percentage of yellow coloration was not correlated with the color score (Spearman $r_s = -0.003$, $P = 0.966$, $n = 187$).

2.2 Parents-offspring regression

Neither the percentages of yellow and red coloration nor the color scores of male offspring differed significantly among sires. These results were expected because we minimized the color variation of the sires by using sires of a population largely fixed for one male color phenotype. A dam's identity, however, had a significant effect on the percentage of red coloration and on the color score in the mixed models (Table 1), as well as in the linear models (Table 2). Additionally, there was a trend in the effect of dam's identity on the percentage of yellow coloration in the male offspring in both models. These results were also expected because the dams were taken from a population that segregates for male coloration. In the mixed model, dam's identity explained 26% of the variation in yellow coloration of the male offspring. Likewise, dams explained 37% of the variation in red coloration of the male offspring. Finally, the color score was also significantly affected by dam's identity, the latter explaining 30% of the variation among male offspring. These percentages are equivalent to heritabilities.

Offspring coloration, expressed in percentage of yellow coloration, percentage of red coloration, and color score, is plotted for each dam in Fig.3. The dams are ranked along the x-axis, from red-preferring females on the left to blue-preferring females on the right.

Table 1 Results of parents-offspring regression models for three measures of male coloration (percentage red, percentage yellow, and color score) with random effects of dams and sires

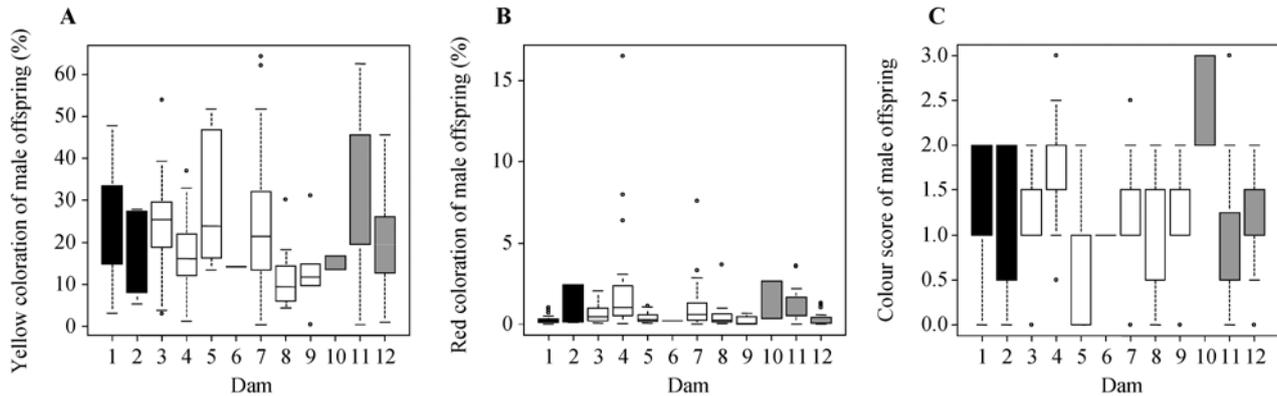
Color measure male offspring	Sire χ^2	<i>df</i>	<i>P</i>	Dam χ^2	<i>df</i>	<i>P</i>	Significant effects in model	<i>r</i> (correlation with mating preference)	<i>P</i>
Yellow (%)	1.050	1	0.306	3.060	1	0.080	Dam	0.084	0.792
Red (%)	0	1	1	9.687	1	0.002	Dam	-0.014	0.974
Color score	0	1	1	4.821	1	0.028	Dam	0.006	0.860

χ^2 test values, degrees of freedom (*df*) and *P*-values are given for the random effects of the dams and sires. Spearman correlations were calculated between pairs of predicted random effect values per dam for a coloration trait and for mating preference.

Table 2 Results of parents-offspring regression models for three measures of male coloration (percentage red, percentage yellow, and color score) with fixed effects of dams and sires

Color measure male offspring	Sire <i>F</i>	<i>df</i>	<i>P</i>	Dam <i>F</i>	<i>df</i>	<i>P</i>	Significant effects in model	<i>r</i> (correlation with mating preference)	<i>P</i>
Yellow (%)	1.711	6	0.121	1.836	11	0.051	Dam	0.126	0.692
Red (%)	0.899	6	0.497	2.432	11	0.007	Dam	0.084	0.792
Color score	0.947	5	0.452	2.546	10	0.007	Dam	0.007	0.983

F-test values, degrees of freedom (*df*) and *P*-values are given for the fixed effects of the dams and sires. Spearman correlations were calculated between pairs of predicted fixed effect values per dam for a coloration trait and for mating preference.

**Fig. 3** Box plot of male offspring coloration of the 12 different dams

The dams are ranked along the x-axis from red-prefering on the left to blue-prefering on the right. Black bars indicate dams with a significant preference for males of the red species, grey bars indicate a significant preference for males of the blue species, and open bars are dams without a preference. A. Yellow coloration (%). B. Red coloration (%). C. Color score. Male color score ranges from 0 (= blue) to 4 (= red).

Table 3 Results of parents-offspring regression bivariate mixed-effect models for female mate preference and male offspring coloration and the heritability of male coloration

Color measure male offspring	χ^2	<i>P</i>	Between-dam variance color	Residual variance color	Heritability
Yellow (%)	0.1	0.75	42.6	163.6	0.82
Red (%)	1.6	0.21	0.27	3.16	0.31
Color score	0.8	0.37	0.039	0.429	0.33

2.3 Correlation between preference and coloration

None of the genotypic color values were correlated with the estimated mating preference of the dam (Spearman correlations in Table 1 and 2, bivariate mixed-effect models in SAS in Table 3). In the bivariate mixed-effect models, heritabilities calculated from the variances between dams and the total variances were 82% of the variation in yellow coloration, 31% of the variation in red coloration, and 32% of the variation in color score.

3 Discussion

Physical linkage between genes affecting mating preferences and sexual traits can enhance the likelihood of speciation because, once evolved, covariance protects against recombination due to random mating (Lande, 1981; Kronforst et al., 2006). Physical linkage has indeed been reported in *Heliconius* butterfly species and

Laupala cricket species (Kronforst et al., 2006; Shaw and Lesnick, 2009). Here, we studied a population of Lake Victoria cichlid fish that contains considerable variation in female mating preference and in male nuptial coloration, the extremes of which resemble differences between sympatric species, with red and blue males, at other locations (Seehausen, 1997; van der Sluijs et al., 2008a; Seehausen, 2009). Females with known preference or known absence thereof were mated with blue males from a closely related species. Body coloration of male offspring was analyzed for potential correlations with the mating preference of its dams. As expected, the identity of the sire had no effect on male offspring coloration. This is consistent with previous data that showed that offspring of two individuals of the blue species have blue-grey coloration (Seehausen, 1997; Seehausen et al., 1997). We found that dam's identity significantly affected the coloration of the off-

spring, suggesting heritable variation in male color genes among females.

However, we found no evidence for any genetic correlation between female mating preference and male nuptial coloration. This does not rule out the existence of weak linkage. Haesler and Seehausen (2005) have shown that female mating preference for red versus blue males in this system is likely codetermined by several genes. The variation in body coloration within our clutches was large, which suggests that this trait is also determined by several genes. When female mating preference and male coloration are both determined by several genes, it may be difficult to detect potential linkage of some of these genes. However, our data rule out strong physical linkage between major effect genes for mating preference and for the preferred trait that would be protected against recombination and aid speciation, as have been observed in *Heliconius* butterflies (Kronforst et al., 2006). The implication is that the sympatric buildup or maintenance of preference/trait covariance in the *Pundamilia* cichlid system requires strong divergent or disruptive selection. Positive correlations between mating preferences and sexual traits have been found in artificial selection experiments in guppies, sticklebacks, and stalk-eyed flies (Houde and Endler, 1990; Bakker, 1993; Houde, 1994; Wilkinson and Reillo, 1994). Furthermore, genetic covariance between female and male components of mate recognition can coevolve rapidly under sexual selection in *Drosophila serrata* × *D. birchii* hybrids (Blows, 1999). In these studies, speciation by sexual selection through mate choice has been suggested, but it is unknown whether speciation was facilitated by genetic linkage of the traits.

We do not know for certain whether the cichlid population at Luanso Island was once differentiated into a blue and a red species that coalesced due to recently increased rates of hybridization or, alternatively, if this population had never been differentiated into two species. Either way, our results imply that random mating and recombination in this turbid-water site have been sufficient to prevent any buildup of, or remove, previously existing genetic correlations between female mating preference and male nuptial coloration. The absence of detectable associations also implies that the persistence of the variation in preference and trait in this population is not explained simply by immigration of individuals from clear-water sites. In other words, the rare red-preferring females at Luanso are not migrants from red populations further north in the Mwanza Gulf.

Further research is necessary to unravel the genetic architecture of female mating preference and male coloration in *Pundamilia* species.

Acknowledgements We thank the Tanzanian Fisheries Research Institute for hosting and use of the facilities (Prof. 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. Peter Dijkstra, Kees Hofker, Charlotte Lindeyer, Martine Maan, John Mrosso, and Machteld Verzijden are greatly acknowledged for collaboration in the field and discussions. Martine Maan and Erin Reardon are also acknowledged for comments on earlier versions of this manuscript. This research was supported by the Netherlands Science Foundation (NWO-ALW 810.64.011), and research grants from Leiden University Fund and the Schure-Beijerinck-Popping Foundation. This study was approved by the Animal Ethics Screening Committee (UDEC 03061).

References

- Andersson M, 1994. *Sexual Selection*. Princeton: Princeton University Press.
- Bakker TCM, 1993. Positive genetic correlation between female preference and preferred male ornament in sticklebacks. *Nature* 363: 255–257.
- Bates D, Sarkar D, 2006. Linear mixed-effects models using s4 classes <http://cran.r-project.org/web/packages/lme4/index.html>.
- Blows MW, 1999. Evolution of the genetic covariance between male and female components of mate recognition: An experimental test. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* 266: 2169–2174.
- Darwin C, 1871. *The descent of man, and selection in relation to sex*. New York: Appleton.
- Dijkstra PD, Seehausen O, Pierotti MER, Groothuis TGG, 2007. Male-male competition and speciation: aggression bias towards differently coloured rivals varies between stages of speciation in a Lake Victoria cichlid species complex. *J. Evol. Biol.* 20: 496–502.
- Dominey WJ, 1984. Effects of sexual selection and life history on speciation: Species flocks in African cichlids and Hawaiian *Drosophila*. In: *Evolution of fish species flocks*. Echelle AA, Kornfield I ed. Orono, ME: University of Maine at Orono Press, 231–249.
- Fisher RA, 1930. *The genetical theory of natural selection*. Oxford: Clarendon.
- Gavrilets S, Vose A, Barluenga M, Salzburger W, Meyer A, 2007. Case studies and mathematical models of ecological speciation. 1. Cichlids in a crater lake. *Molec. Ecol.* 16: 2893–2909.
- Genner MJ, Turner GF, 2005. The mbuna cichlids of Lake Malawi: A model for rapid speciation and adaptive radiation. *Fish and Fisheries* 6: 1–34.
- Gueorguieva R, 2001. A multivariate generalized linear mixed model for joint modelling of clustered outcomes in the exponential family. *Stat. Modelling* 1: 177–193.
- Haesler MP, Seehausen O, 2005. Inheritance of female mating preference in a sympatric sibling species pair of Lake Victoria cichlids: implications for speciation. *Proc. R. Soc. Lond. Ser. B. Sci.* 272: 237–245.

- Higashi M, Takimoto G, Yamamura N, 1999. Sympatric speciation by sexual selection. *Nature* 402: 523–526.
- Houde AE, 1994. Effect of artificial selection on male color patterns on mating preference of female guppies. *Proc. R. Soc. Lond. Ser. B. Sci.* 256: 125–130.
- Houde AE, Endler JA, 1990. Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. *Science* 248: 1405–1408.
- Ihaka R, Gentleman R, 1996. R: A language for data analysis and graphics. *J. Comput. Graph. Stat.* 5: 299–314.
- Kirkpatrick M, 1982. Sexual selection and the evolution of female choice. *Evolution* 36: 1–12.
- Knight ME, Turner GF, 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. Ser. B-Biol. Sci.* 271: 675–680.
- Kocher TD, 2004. Adaptive evolution and explosive speciation: The cichlid fish model. *Nat. Genet.* 5: 288–298.
- Kornfield I, Smith PF, 2000. African cichlid fishes: Model systems for evolutionary biology. *Annu. Rev. Ecol. Syst.* 31: 163–196.
- Kronforst MR, Young LG, Kapan DD, McNeely C, O'Neill RJ, et al., 2006. Linkage of butterfly mate preference and wing color preference cue at the genomic location of wingless. *Proc. Natl. Acad. Sci. U. S. A.* 103: 6575–6580.
- Lande R, 1981. Models of speciation by sexual selection on polygenic traits. *Proc. Natl. Acad. Sci. U. S. A.* 78: 3721–3725.
- Lynch M, Walsh B, 1997. Genetics and analysis of quantitative traits. Sunderland, MA: Sinauer Associates.
- Maan ME, Seehausen O, Soderberg L, Johnson L, Ripmeester EAP et al., 2004. Intraspecific sexual selection on a speciation trait, male coloration, in the Lake Victoria cichlid *Pundamilia nyererei*. *Proc. R. Soc. Lond. Ser. B. Sci.* 271: 2445–2452.
- Maan ME, van der Spoel M, Jimenez PQ, van Alphen JJM, Seehausen O, 2006. Fitness correlates of male coloration in a Lake Victoria cichlid fish. *Behav. Ecol.* 17: 691–699.
- Moller AP, Jennions MD, 2001. How important are direct fitness benefits of sexual selection? *Naturwissenschaften* 88: 401–415.
- Pauers MJ, McKinnon JS, Ehlinger TJ, 2004. Directional sexual selection on chroma and within-pattern colour contrast in *Labeotropheus fuelleborni*. *Proc. R. Soc. Lond. Ser. B. Sci.* 271: S444–S447.
- Payne RJH, Krakauer DC, 1997. Sexual selection, space, and speciation. *Evolution* 51: 1–9.
- Saetre GP, Fossnes T, Slagsvold T, 1995. Food provisioning in the pied flycatcher: Do females gain direct benefits from choosing bright-colored males? *J. Anim. Ecol.* 64: 21–30.
- Seehausen O, 1996. Lake Victoria Rock Cichlids: Taxonomy, Ecology and Distribution. Zevenhuizen: Verduijn.
- Seehausen O, 1997. Distribution of and reproductive isolation among color morphs of a rock-dwelling Lake Victoria cichlid *Haplochromis nyererei* (vol 5, pg 195, 1996). *Ecol. Freshw. Fish* 6: 57–66.
- Seehausen O, 2006. African cichlid fish: a model system in adaptive radiation research. *Proc. R. Soc. Lond. Ser. B. Sci.* 273: 1987–1998.
- Seehausen O, 2009. Progressive levels of trait divergence along a 'speciation transect' in the Lake Victoria cichlid fish *Pundamilia*. In: Butlin R, Schluter D, Bridle JR ed. *Speciation and Patterns of Diversity*. Cambridge, UK: Cambridge University Press, 155–176.
- 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, Terai Y, Magalhaes IS, Carleton KL, Mrosso HDJ et al, 2008. Speciation through sensory drive in cichlid fish. *Nature* 455: 620–627.
- Seehausen O, van Alphen JJM, 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 JJM, Witte F, 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* 277: 1808–1811.
- Shaw KL, Lesnick SC, 2009. Genomic linkage of male song and female acoustic preference QTL underlying a rapid species radiation. *Proc. Natl. Acad. Sci. U. S. A.* 106: 9737–9742.
- Stelkens RB, Pierotti MER, Joyce DA, Smith AM, van der Sluijs I et al., 2008. Disruptive sexual selection on male nuptial coloration in an experimental hybrid population of cichlid fish. *Philos. Trans. R. Soc. Lond. Ser. B. Sci.* 363: 2861–2870.
- Taylor MI, Turner GF, Robinson RL, Stauffer JR, 1998. Sexual selection, parasites and bower height skew in a bower-building cichlid fish. *Anim. Behav.* 56: 379–384.
- Turner GF, Burrows MT, 1995. A model of sympatric speciation by sexual selection. *Proc. R. Soc. Lond. Ser. B. Sci.* 260: 287–292.
- van der Sluijs I, van Alphen JJM, Seehausen O, 2008a. Preference polymorphism for coloration but no speciation in a population of Lake Victoria cichlids. *Behav. Ecol.* 19: 177–183.
- van der Sluijs I, Van Dooren TJM, Hofker KD, van Alphen JJM, Stelkens RB et al., 2008b. Female mating preference functions predict sexual selection against hybrids between sibling species of cichlid fish. *Philos. Trans. R. Soc. Lond. Ser. B. Sci.* 363: 2871–2877.
- van der Sluijs I, Van Dooren TJM, Seehausen O, van Alphen JJM, 2008c. A test of fitness consequences of hybridization in sibling species of Lake Victoria cichlid fish. *J. Evol. Biol.* 21: 480–491.
- van Doorn GS, Dieckmann U, Weissing FJ, 2004. Sympatric speciation by sexual selection: A critical reevaluation. *Am. Nat.* 163: 709–725.
- Van Oppen MJH, Turner GF, Rico C, Robinson RL, Deutsch JC et al., 1998. Assortative mating among rock-dwelling cichlid fishes supports high estimates of species richness from Lake Malawi. *Mol. Ecol.* 7: 991–1001.
- Wilkinson GS, Reillo PR, 1994. Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly. *Proc. R. Soc. Lond. Ser. B. Sci.* 255: 1–6.
- 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.
- Wu CI, 1985. A stochastic simulation study on speciation by sexual selection. *Evolution* 39: 66–82.
- Zahavi A, 1975. Mate selection: A Selection for a handicap. *J. Theor. Biol.* 53: 205–214.