Inheritance of female mating preference in a sympatric sibling species pair of Lake Victoria cichlids: implications for speciation

Marcel P. Haesler† and Ole Seehausen*

Molecular and Evolutionary Ecology Group, Department of Biological Sciences, University of Hull, Hull HU6 7RX, UK

Female mate choice has often been proposed to play an important role in cases of rapid speciation, in particular in the explosively evolved haplochromine cichlid species flocks of the Great Lakes of East Africa. Little, if anything, is known in cichlid radiations about the heritability of female mating preferences. Entirely sympatric distribution, large ecological overlap and conspicuous differences in male nuptial coloration, and female preferences for these, make the sister species *Pundamilia pundamilia* and *P. nyererei* from Lake Victoria an ideally suited species pair to test assumptions on the genetics of mating preferences made in models of sympatric speciation. Female mate choice is necessary and sufficient to maintain reproductive isolation between these species, and it is perhaps not unlikely therefore, that female mate choice has been important during speciation. A prerequisite for this, which had remained untested in African cichlid fish, is that variation in female mating preferences is heritable. We investigated mating preferences of females of these sister species and their hybrids to test this assumption of most sympatric speciation models, and to further test the assumption of some models of sympatric speciation by sexual selection that female preference is a single-gene trait. We find that the differences in female mating preferences between the sister species are heritable, possibly with quite high heritabilities, and that few but probably more than one genetic loci contribute to this behavioural speciation trait with no apparent dominance. We discuss these results in the light of speciation models and the debate about the explosive radiation of cichlid fishes in Lake Victoria.

Keywords: cichlid fish; genetics of reproductive isolation; female mating preference; Lake Victoria sexual selection; sympatric speciation

1. INTRODUCTION

Female mate choice can be a powerful force of selection on male secondary sexual characteristics in animals (Andersson 1994), and a potential agent of rapid population differentiation and speciation (Fisher 1930; West Eberhard 1979; Lande 1981*b*, 1982). Several theoretical models suggest that, if disruptive, selection exerted by female choice could cause speciation in the absence of geographical isolation (Lande 1982; Wu 1985; Turner & Burrows 1995; Payne & Krakauer 1997; van Doorn *et al.* 1998, 2004; Higashi *et al.* 1999; Kawata & Yoshimura 2000; Takimoto *et al.* 2000; Takimoto 2002; van Doorn *et al.* 2004). The conditions for this to happen in complete sympatry are stringent, although speciation by sexual selection along a cline is less controversial (Endler 1977; Lande 1982; Kirkpatrick & Ravigné 2002).

A textbook case of multiple and rapid speciation in which female mate choice has been invoked, is the explosive adaptive radiation of haplochromine cichlids in Lake Victoria. More than 500 species appear to have evolved from very few ancestral species within perhaps as little as

15 000 years (Johnson *et al.* 1996, 2000; but see Fryer 2004). Sexual selection has been proposed as a major player in this rapid diversification, as well as in the radiation of haplochromine cichlids in Lake Malawi (Dominey 1984; McKaye 1991; Seehausen *et al.* 1997; Galis & Metz 1998; Turner 1998; Kornfield & Smith 2000; Kocher 2004). Closely related sympatric species in these species flocks are often ecologically very similar (Bouton *et al.* 1997, 1998; Seehausen & Bouton 1997; Genner *et al.* 1999) but differ strongly in male nuptial coloration (Albertson *et al.* 1999; Seehausen & van Alphen 1999; Smith & Kornfield 2002; Allender *et al.* 2003). A previously studied example is the sympatric sibling species pair *Pundamilia nyererei* and *P. pundamilia* from Lake Victoria. These completely sympatric species are at most localities reproductively isolated by female mate choice (Seehausen 1997; Seehausen & van Alphen 1998). Given that the geographical range of *P. nyererei* is completely nested within the larger range of *P. pundamilia* (Seehausen & van Alphen 1999), and that female mate choice is sufficient and necessary for behavioural reproductive isolation between them (Seehausen & van Alphen 1998), this pair would seem one of the best candidate cases for sympatric speciation through divergent mate choice.

Several modelling approaches suggest that the likelihood of sympatric speciation through divergent mate choice critically depends on the genetic architecture of female mating preferences. In models that start from a monomorphic condition to which a new preference mutant and a

*Author and address for correspondence: Zoological Institute, University of Bern, Baltzerstrasse 6, CH-3012 Bern, and Limnological Research Centre, EAWAG, Seestrasse 79, CH-6047 Kastanienbaum, Switzerland. (ole.seehausen@aqua.unibe.ch).
† Present address: Zoological Institute, Department of Behavioural Ecology, University of Bern, Wohlenstrasse 50a, CH-interkappelen, Switzerland.

matching male trait mutant are introduced at low frequencies, the likelihood of sympatric speciation by divergent mating preferences decreases as the number of loci controlling female mating preference increases above one (Lande 1982; Arnegard & Kondrashov 2004). In fact, models of speciation where female choice is the only source of disruptive selection in complete sympatry, assume an oligogenic nature of female mating preferences: one (Turner & Burrows 1995; Takimoto 2002) or two loci (Payne & Krakauer 1997).

Sister species pairs of Lake Victoria and Lake Malawi haplochromines often consist of a species with yellow-red and one with blue male nuptial dress (Seehausen 1996; Seehausen *et al.* 1997; Smith & Kornfield 2002; Allender *et al.* 2003). We investigated the genetic basis of female mating preferences for red and blue male nuptial coloration in the sibling species pair *P. nyererei* (red) and *P. pundamilia* (blue). We studied mate preferences of F_1 and F_2 hybrid females as well as parental type females to test the hypotheses that female mate preference is (i) heritable and (ii) determined by a single genetic locus. Although reproductive isolation in the wild is not complete in the population that we studied (Seehausen 1997), behavioural mate choice of females of both species is species assortative when they can see the male nuptial coloration but not when the differences among the latter are masked by monochromatic light (Seehausen & van Alphen 1998). Similarly, random mating in turbid water, but assortative mating in clear water, had been inferred from phenotype frequency distributions in nature (Seehausen *et al.* 1997). If female mating preferences are heritable, we expect the first hybrid generation females to express relatively uniform mating preferences, whereas preferences should segregate again among second hybrid generation females. This is the first attempt, to our knowledge, to characterize the genetic basis of divergent female mating preferences in the African radiations of cichlid fish. Given the nested geographical distribution, non-zero gene flow, the divergent female preferences and their effect on male colour variation in clear waters, the species pair that we use is ideally suited for testing genetic assumptions of models of sympatric speciation through divergent mating preferences.

2. MATERIAL AND METHODS

(a) *The species and their behaviour*

Males and females of *P. pundamilia* and *P. nyererei* are morphologically highly similar but *P. nyererei* males have a red dorsum and yellow flanks, whereas those of *P. pundamilia* are grey/blue. The females can only be distinguished with considerable experience. Courtship behaviour resembles that of other Lake Victoria haplochromines. For a description of the courtship behaviour, see Seehausen & van Alphen (1998), with photographs and drawings in Seehausen (1996). We recorded the following elements of male courtship behaviour: 'lateral display' (LD), 'quiver' (QU) and 'lead swim' (LE). Each courtship bout began either with an 'approach' (AP)–LD sequence or directly with LD. Recorded elements of female courtship, i.e. positive response to male courtship, were AP (upon male courtship) and 'follow' (FO, upon male lead swimming).

All males and non-hybrid females were the same laboratory stock used to demonstrate species-assortative female mating preferences earlier (Seehausen & van Alphen 1998), derived from

the Python Islands in the Mwanza Gulf of Lake Victoria, except four stimulus red males that were caught from Kissenda Island, also in the Mwanza Gulf. The populations from Python and Kissenda Islands are phenotypically indistinguishable. Hybrids can be easily bred in aquaria by keeping females of one species with males of the other one. They are fully viable and fertile through several generations (Seehausen *et al.* 1997). F_1 hybrids were bred from *P. nyererei* males (red) and *P. pundamilia* females (blue) and vice versa. F_2 hybrids were bred from randomly chosen F_1 hybrids.

(b) *Housing conditions and experimental set-up*

Experiments were conducted in two series, one in 2000 (series 1) and the other in 2001–2002 (series 2). Before the experiments, males and females were kept separately in a large recirculation aquarium system, maintained at 24–26 °C, on a 12 L : 12 D cycle. The tanks were illuminated with 40 W daylight fluorescent tubes. Experimental tanks measured 200 cm × 50 cm × 40 cm, filled up with sand to a depth of 3 cm. At each end of the tank, a cave was built from three to five rocks. One red male was placed at one end of the tank and one blue male at the other end. The caves were dark inside but open to the observer, who could see into them. In the middle of the tank was an additional smaller cave for the female to allow her to settle in quickly when introduced into the experimental tank.

The F_2 females had no previous mating experience. They were raised in mono-sex groups from the moment that the sexes became distinguishable. F_1 females had prior mating experience exclusively with F_1 males. All experiments were free-contact experiments (Turner *et al.* 2001). Males were introduced several days, and females 5 min before, a trial was started. Males were separated from each other and from a large central section of the tank by plastic grids. This was so that males could not interact with each other. In the first experiment series (2000), the grids were removed for the duration of a trial. In the second series (2001 and 2002), we left them in place during the trials and adjusted the mesh size of the grids such that the females could easily move through but the males were retained in their sections. Because the males had much deeper bodies, this was easily achieved. We tried to match red and blue males by size (standard length) and mass. Only females with ripe ovaries were chosen for trials. Gravidity was determined by swelling of abdomen and urogenital opening and was scored on a 5-point scale (Seehausen & van Alphen 1998). Only females with a minimum score of 3–4 were used.

(c) *Determination of female mating preferences*

After a pair of males had established itself and begun to show nuptial coloration and display territorial behaviour, every female within one of the two experiment series was tested with that pair. Each trial lasted 15 min. Such a trial series was completed when every female had been successfully tested with the same male pair. Then the males were exchanged against a new pair. Trials with the new male pair were started as soon as the males showed nuptial coloration and territorial behaviour around their caves. Fifty females were tested with six, 15 with five, and one with four different male pairs. A trial was successful and used for statistical analysis if the following minimum requirements were fulfilled: five or more encounters of the female with each male, plus two or more lateral displays of each male to the female, plus one or more response by the female to at least one male within 15 min. If these minimum requirements were not met, the trial was discarded and repeated at a later stage. If a female hid continuously for 5 min or longer, the trial was extended for 5 min to keep the length of the
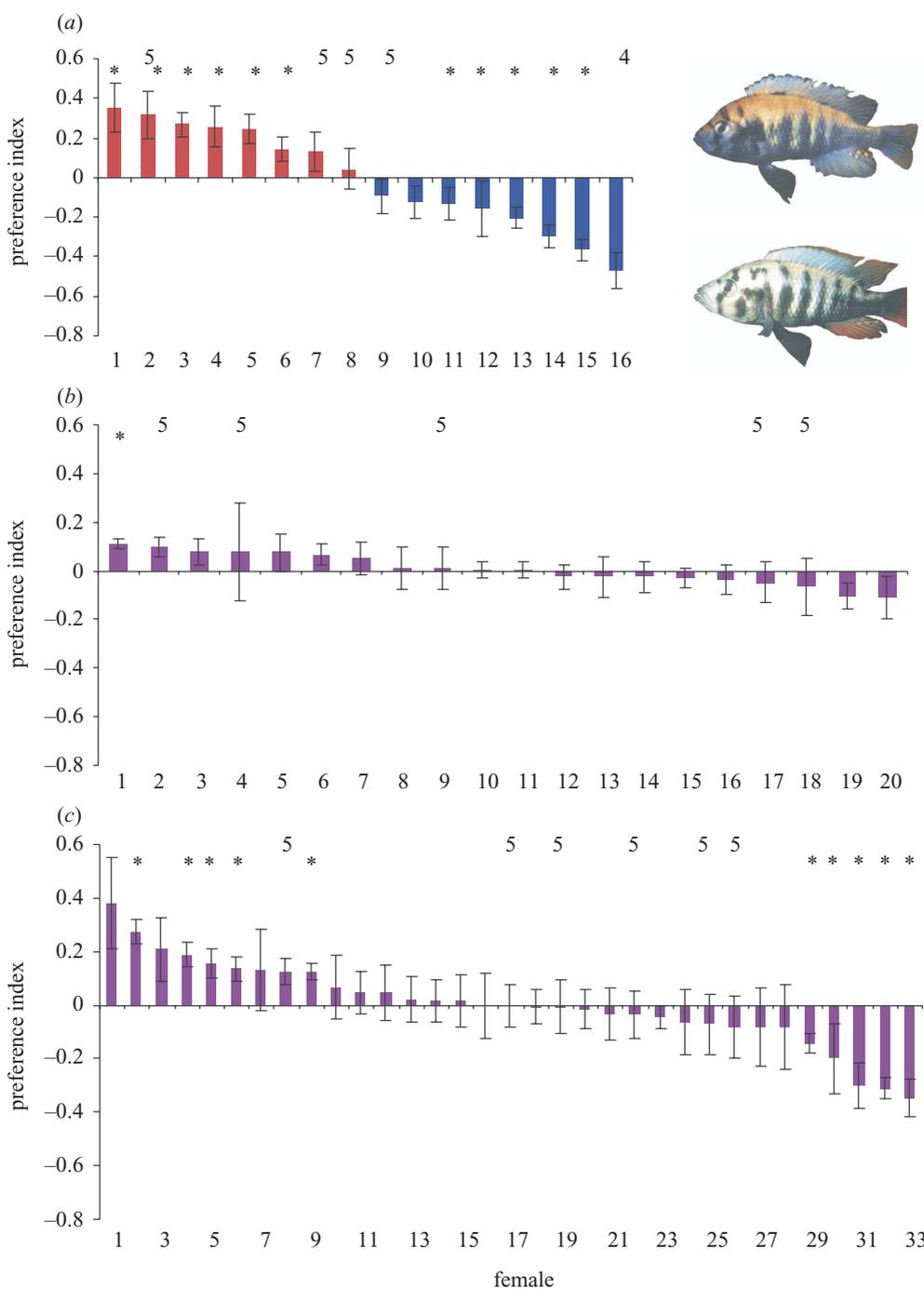
Figure 1. Mean preference indices and standard errors of all females tested. *Significant preferences ($p < 0.05$). Each female was tested with six different male pairs unless when indicated otherwise above the bars. (*a*) Parental-type female preferences, and one example of each of the two male colour phenotypes; (*b*) F_1 hybrid female preferences; (*c*) F_2 hybrid female preferences.

observed female–male interaction period at *ca.* 15 min. A total of 379 successful trials were conducted. The frequency of female–male encounters with male courtship display (LD) was quantified as a proportion of all female–male encounters. Female mating preference was measured as the proportion of courtship events of the red male that elicited a positive female response minus the proportion of courtship events of the blue male that elicited a positive female response. We refer to this as a preference index.

(d) *Testing whether female preferences are heritable and biometric estimation of number of genes*
If female mating preferences are heritable, and assuming the parental lines were highly homozygous for alternative alleles at their

preference loci, the F_1 hybrids should be genetically uniform and preference variation observed among them would be environmentally induced or measurement error. As preference genes would segregate in the F_2 hybrids, genetic variance adds to the environmental or measurement variance. None of these predictions would be satisfied if preference were not heritable. We used the Castle–Wright estimator (Castle 1921; Wright 1968; Lande 1981*a*) to biometrically estimate the minimum number of genes contributing to the difference in female mating preference between the species. After subtraction of the variance observed among F_1 hybrids and parental lines, the remaining variance in the F_2 is a conservative estimate of the heritable component of vari-

ance. From the amplitude between the means of the parental lines and the genetic variance (segregating variance, σ_S) in the F_2 hybrids, the number of unlinked genes (= minimum number of genes) controlling the measured trait can be inferred (Lande 1981*a*).

$$n_E = \Delta^2 \Big/ 8\left(\sigma^2_{F_2} - \left[\frac{1}{2}\sigma^2_{F_1} + \frac{1}{4}\sigma^2_{P_1} + \frac{1}{4}\sigma^2_{P_2}\right]\right),$$

$$n_E = \frac{\Delta^2}{8\sigma^2_s},$$

where n_E is the estimate for the minimum number of genes, Δ is the difference between the means of the parental lines and $\sigma^2_{F_2}$ and $\sigma^2_{F_1}$ are the variances of the second and first hybrid generations, respectively, and $\sigma^2_{P_1}$ and $\sigma^2_{P_2}$ are the variances of the two parental lines.

(e) Data analysis

If males courted conspecific females preferentially, this could influence female mate choice. For males of each species separately, we therefore compared the mean proportions of encounters with conspecific and heterospecific females in which the male courted, using a Wilcoxon matched-pairs test, and we compared the mean between the two species by a Mann–Whitney U-test. We further asked whether individual females received more courtship displays from conspecific than from heterospecific males, by comparing for each female the proportion of encounters with red and blue males in which she was courted, with a Wilcoxon matched-pairs test (pairs are males in the same trial).

We measured a female's preference as the difference in her responsiveness to courtship from the red male and the blue male. We used the Wilcoxon matched-pairs test to determine for each female whether the raw data suggested a significant preference. To control for effects of variation in male courtship activity, we calculated residual preferences, obtained by regressing female preference against the difference in courtship activity between red and blue males. We then used a one-sample t-test on the residual preference to determine for each female whether a preference remained significant after variation in male courtship activity was controlled for. These residual preferences were used for all further analysis. To compare the variances in the two hybrid generations, we used an F-test. To test for differences between groups of females (e.g. *P. nyererei* versus *P. pundamilia*), we used a Mann–Whitney U-test on mean preferences of females calculated over all trials with a female. To combine the 2000 and 2001–2002 datasets Fisher's combined p and Bonferroni corrections were used to correct for multiple testing.

We calculated repeatability of interspecific female mating preferences using the parental type females, as $R = \sigma_W{}^2/(\sigma_W{}^2 + \sigma_E{}^2)$, where $\sigma_W{}^2$ is the variance between individuals and $\sigma_E{}^2$ is the variance within an individual (Becker 1992). All tests were done using SPSS 10.0 and MINITAB release 13.

3. RESULTS

(a) Males court females of both species equally

Each of 22 males was tested with three or four females of each species. Males of both species exhibited no bias in courtship effort directed to females of either species (*P. nyererei* males: $Z = -1.067$, $n = 11$, $p = 0.286$; *P. pundamilia* males: $Z = -0.178$, $n = 11$, $p = 0.859$), and there was no difference between males of the two species in their direction of courtship effort towards females of the two species ($n_1 = 11$, $n_2 = 11$, $U = 59$, $p = 0.949$). One of

16 females (*P. nyererei* female R10) received significantly more courtship from conspecific males (Wilcoxon matched-pairs test: $n = 6$, $Z = -2.201$, $p = 0.028$; all others $p \geqslant 0.13$), none received significantly more courtship from heterospecific males.

(b) Female mating preferences in Pundamilia nyererei and P. pundamilia

(i) Repeatability of preference measurements

Repeatabilities of individual female mating preferences in the parental lines were $R = 0.78$ in experiment series 1 ($n = 8$), and $R = 0.50$ in series 2 ($n = 8$), suggesting a maximum for possible heritabilities between 50% and 78%. Pooled over both series we obtained a repeatability of $R = 0.59$ ($n = 16$).

(ii) Female mating preferences differ between the sibling species

Females of *P. nyererei* and *P. pundamilia* exhibited highly significantly different mating preferences ($n_1 = 8$, $n_2 = 8$, $U = 0$, $p = 0.001$; figure 1*a*), consistent with results of earlier investigations that used the same laboratory stock derived from the same populations (Seehausen 1997; Seehausen *et al.* 1997; Seehausen & van Alphen 1998).

(c) Hybrid female mating preferences

Only 1 out of 20 F_1 hybrid females (as expected by chance at $\alpha = 0.05$) exhibited a significant preference (one-sample t-test, $p = 0.002$; figure 1*b*). None of the other 19 exhibited mating preferences ($p \geqslant 0.1$). The mean preference of the F_1 hybrids was intermediate to those of the two parental species, and the variance among F_1 females was small and also intermediate to those among the two parental species (table 1). Nine out of 33 F_2 hybrid females (seven more than expected by chance at $\alpha = 0.05$) exhibited significant preferences (figure 1*c*; one-sample t-test, $p \leqslant 0.05$). The mean preference of F_2 females was similar to that of F_1 females, but the variance was significantly higher in F_2 females (F-test: experiment series 1: $n_1 = 15$, $n_2 = 11$, $F = 0.068$, $p < 0.001$; experiment series 2: $n_1 = 5$, $n_2 = 22$, $F = 0.625$, $p = 0.701$; combined dataset: $n_1 = 20$, $n_2 = 33$, $F = 0.167$, $p < 0.001$; table 2).

(i) Relationship between strength and consistency of female preference

We calculated the coefficient of variation (CV) by dividing the variance by the mean, to compare the variances in the different groups of females (i.e. parental-type, F_1 and F_2 hybrids). A strong correlation emerged between the sign-removed mean and its CV (figure 3). The weaker the mean preference of a female for either red or blue males, the larger is the variation in her response measured over different male pairs. The CV for F_2 hybrid females is greater than that for F_1 hybrid females and both parental types, confirming greater variance in the F_2 hybrid females (mean $CV_{F_2} = 2.33$; mean $CV_{F_1} = 1.07$; mean $CV_{nyererei} = 0.38$; mean $CV_{pundamilia} = 0.25$).

(ii) Inheritance of female mating preference

The mating preferences of females in both hybrid generations are intermediate to those in females of the parental populations, and the variance in the second hybrid

Table 1. Means and variance of residual mating preference for the four female genotype classes.

female genotype classes	experiment 2000			experiment 2001–2002			2000 and 2001–2002 pooled		
	n	mean	variance	n	mean	variance	n	mean	variance
red	4	0.2985	0.0021	4	0.1413	0.0071	8	0.2199	0.0109
blue	4	−0.1689	0.0169	4	−0.1398	0.0021	8	−0.2321	0.0178
F_1 hybrids	15	0.0031	0.0041	5	0.0140	0.0052	20	0.0058	0.0041
F_2 hybrids	11	<0.0001	0.0627	22	0.0003	0.0076	33	0.0030	0.0250

Table 2. Results of Mann–Whitney U-tests comparing the different female genotype classes.

experiment 2000	n_1, n_2	Z	p	experiment 2001–2002	n_1, n_2	Z	p
red versus blue	4, 4	−2.309	0.029	red versus blue	4, 4	−2.309	0.029
F_1 versus red	15, 4	−3	0.003	F_1 versus red	5, 4	−1.960	0.050
F_1 versus blue	15, 4	−3	0.003	F_1 versus blue	5, 4	−2.449	0.014
F_2 versus red	11, 4	−3	0.003	F_2 versus red	22, 4	−2.416	0.016
F_2 versus blue	11, 4	−3	0.003	F_2 versus blue	22, 4	−2.914	0.004
F_2 versus F_1	11, 15	0	1	F_2 versus F_1	22, 5	−0.375	0.708

experiment 2000 and 2001–2002 pooled	n_1, n_2	Fisher's combined p	Bonferroni corrected p
red versus blue	8, 8	0.00512	0.01024
F_1 versus red	8, 20	0.00055	0.00218
F_1 versus blue	8, 20	0.00017	0.00102
F_2 versus red	8, 33	0.00156	0.00468
F_2 versus blue	8, 33	0.00045	0.00223
F_2 versus F_1	20, 33	0.83491	0.83491

generation is greater than in parental females and in the first hybrid generation (figures 1 and 2). This, and the relationship between strength and consistency of preferences, are strong evidence for heritability of the interspecific difference in female mating preferences.

(iii) *Estimation of minimum number of genes*

The minimum number of genes contributing to the difference in female preference between *P. nyererei* and *P. pundamilia* for red versus blue males was calculated from all females pooled, as well as for the two experiment series separately. Using the residual preferences, the estimates are one gene (series 1) and four genes (series 2). With raw data, not corrected for effects of variation in male courtship, we obtained one and five genes, respectively. To pool the data from the two experiment series that differed in the amplitude of observed between-female variation in preference, we calculated, using the raw preferences, for each series the ratio (Q) as

$$Q = \frac{D_{R-B}}{D_{F_2,\text{max}-\text{min}}},$$

where D_{R-B} is the difference between the mean preferences of the two parental species, and $D_{F_2,\text{max}-\text{min}}$ is the difference between the highest and the lowest preferences of F_2 hybrid females. Series 1 yielded $D_{R-B} = 0.80$, $D_{F_2,\text{max}-\text{min}} = 1.08$ and a ratio $Q = 0.735$; series 2 yielded $D_{R-B} = 0.29$, $D_{F_2,\text{max}-\text{min}} = 0.40$ and $Q = 0.747$. Given that the ratios were essentially identical, we calculated an adjustment factor (F) as the ratio of D_{R-B} of the two series, i.e. $F = 0.29/0.80 = 0.36$. We then calculated adjusted

preferences for the females of series 1 by multiplying their mean preference indices with F. The pooled data using all 16 parental, 20 F_1 and 33 F_2 hybrid females then yielded an estimate of 1.6 for the minimum number of genes.

4. DISCUSSION

Our data strongly suggest that the difference in female mating preferences for different male nuptial dress between the sympatric closely related, and occasionally hybridizing, cichlid species *Pundamilia pundamilia* and *P. nyererei* is heritable. We hybridized laboratory lines of these species that exhibited significantly different preferences. The mean preference of females in the first and second hybrid generations was intermediate to those of females of the two parental species. The means of F_1 and F_2 hybrid generations were not significantly different from each other, but the variances differed, with the F_2 generation significantly more variable than the F_1 generation. Hence, female preference behaved as expected from a heritable trait. High repeatabilities of parental type preferences are consistent with potentially high heritabilities for the interspecific difference in preferences (Falconer & Mackay 1996).

Males did not direct courtship effort preferentially to either conspecific or heterospecific females. Therefore, we conclude that (i) males did not differentiate between females of the two species and (ii) preferences of *P. pundamilia* and *P. nyererei* females cannot be explained by differential male courtship effort, consistent with an earlier study (Seehausen & van Alphen 1998). In experiments of series 1, hybrid females of both generations tended to prefer blue males, whereas hybrid females in the experiments of series 2
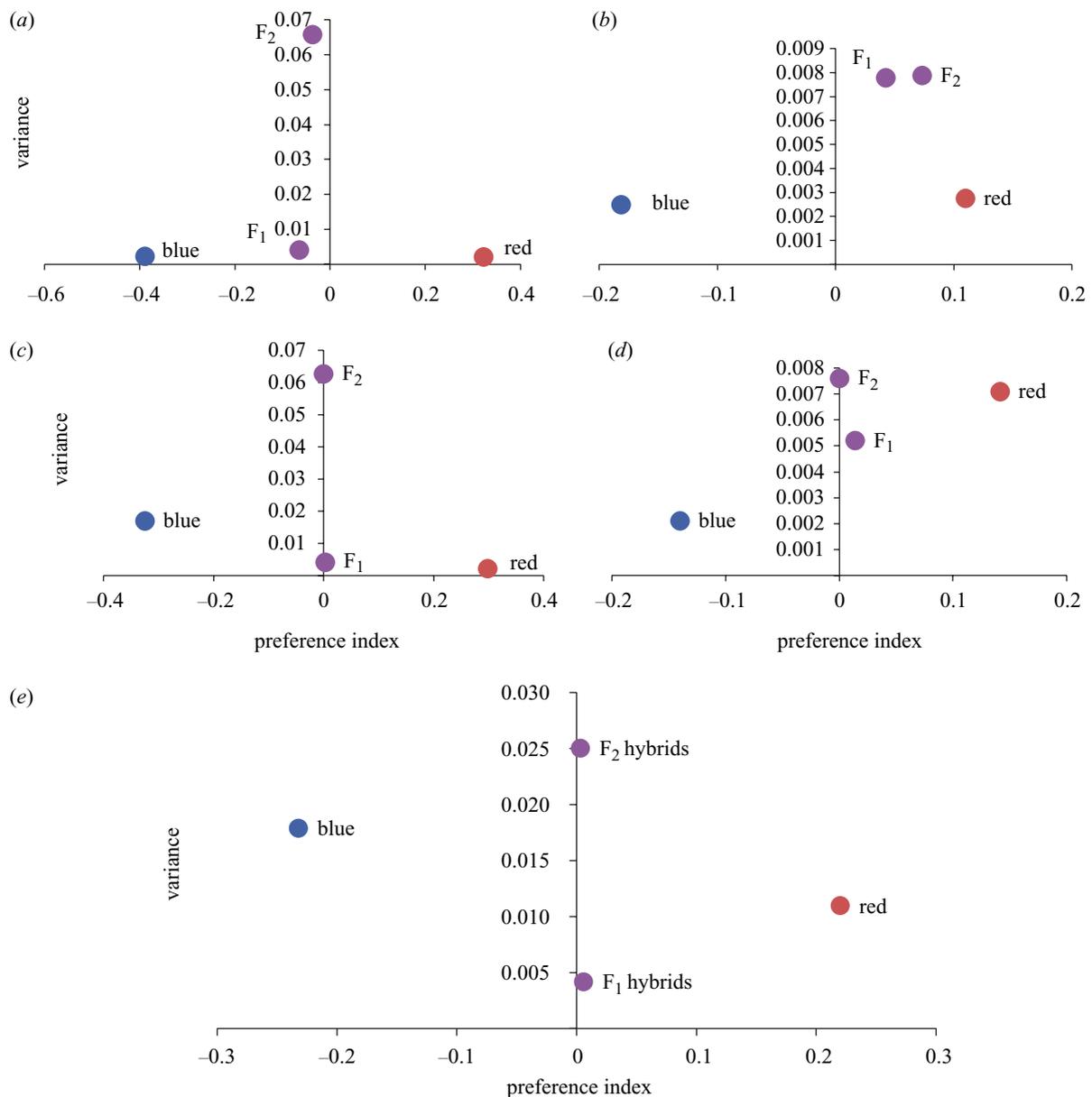
Figure 2. Mean preference index (x-axis) and variance (y-axis) for parental type, F_1 hybrid and F_2 hybrid females. (a) Experiment series 1: raw preferences; (b) experiment series 2: raw preferences; (c) series 1: residual preferences; (d) series 2: residual preferences; (e) both experiment series combined: residual preferences.

tended to prefer red males (figure 2a,b). However, this was largely explained by differences between red and blue males in their mean courtship effort. When differences in male courtship effort were controlled, the means of the female preference indices for both hybrid generations and both experiment series converged on zero (= no preference), whereas those of parental type females were little affected (figure 2c,d).

To obtain accurate estimates of minimum numbers of genes determining a trait difference, Lande (1981a) estimated that a minimum of 20 F_1 and 100 F_2 hybrid phenotypes are required. Our sample sizes are small with 20 F_1 and only 33 F_2 phenotypes. Estimates from small sample sizes are likely to vary greatly around the true minimum number of genes. Nevertheless, we feel that it is justified to conclude from our data that the minimum number of genes contributing to the difference in female mating preferences for red versus blue males in this sympatric species pair is

small. Our estimates suggest between one and five unlinked loci are involved. The biometrical method tends to underestimate the true number of loci if the assumptions of unlinked loci and equal allelic effects at all loci are violated (Zeng *et al.* 1990). Hence, it seems likely that the true number of genes in our case is larger than 1.

Our data suggest that preference alleles are largely additive, although the possibility of dominance on some preference loci that could cancel each other out cannot be discarded. Our data further highlight the danger of arriving at the wrong conclusions if male behaviour is not controlled for when female preferences are measured. This is especially obvious in heterozygous preference genotypes that have no strong preference for specific male traits of either species and are hence a lot more affected by random variation in male courtship behaviour (figure 2).

Colour vision is important in cichlid communication (Evans & Norris 1996; Seehausen *et al.* 1997; Carleton
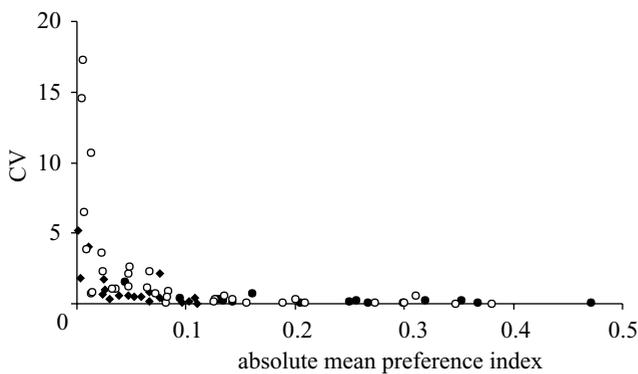
Figure 3. Relationship between strength and consistency of female mating preference: absolute mean preference index and its coefficient of variation (CV) for all females (16 parental type, filled circles; 20 F_1 hybrids, filled diamonds; and 33 F_2 hybrids, open circles). A high CV reflects lack of consistency in relative response to red and blue males; a high absolute mean preference index reflects a strong mean mating preference.

et al. 2004) but simultaneously also plays an important role in food selection (Van der Meer & Bowmaker 1995). Polygenic determination may be expected if multiple selection forces operate on a trait. It is perceivable that a switch in visual colour preference can be achieved with moderate modifications to the visual system, for example through up- or down-regulation of opsin gene expression (Carleton & Kocher 2001). However, mate preferences could also be determined at higher levels of information processing, making it difficult to predict whether species differences should be polygenic or oligogenic. Two studies on genetics of female mating preference in *Drosophila* found a small number of loci determining mating preference (three loci in Ting *et al.* (2001), and one locus or one cluster of loci in Doi *et al.* (2001)). Ritchie (2000) found that the genetic control of mate choice in the bushcricket *Ephippiger ephippiger* could be explained by a simple polygenic additive model. Certainly, the *Drosophila* results compare favourably with ours.

Given the geographical distribution of the sister species *P. nyererei* and *P. pundamilia*, their ecological overlap and only very slight morphological differentiation, but conspicuously different male nuptial colours and female preferences for these, it had been hypothesized that this species pair diverged sympatrically (Seehausen 1997), possibly through disruptive sexual selection (Seehausen & van Alphen 1998). Speciation under disruptive selection on the mating system can potentially be faster than allopatric speciation, where reproductive isolation would have to evolve as a by-product of drift, natural or sexual selection in different habitats (Coyne 1992; Coyne & Orr 1998). Mathematical and simulation models of sympatric speciation, driven by disruptive sexual selection alone, where divergent mating preferences are not reinforced by natural selection against intermediates, fall largely into two categories in terms of the assumptions and conclusions about the genetics of female mating preferences. First, models that begin from monomorphic conditions and introduce both the alternative (novel) mating preference and mating trait as rare alleles, tend to require that mating preferences are determined by just one or two genes (Wu 1985; Turner & Burrows 1995; Payne & Krakauer 1997; Takimoto 2002;

Arnegard & Kondrashov 2004). Although our estimate of between one and five genes is a minimum estimate, we cannot rule out that this is the case in *Pundamilia*, one of the most species-rich genera of Lake Victoria cichlids with one of the highest sympatry indices among East African cichlid species complexes (Genner *et al.* 2004).

Second, in models of speciation from polymorphic populations that segregate for alternative preference and trait alleles at similar and high frequencies, the number of loci has no inhibiting effect on sympatric speciation by disruptive Fisherian runaway selection (Higashi *et al.* 1999; Takimoto *et al.* 2000; Kawata & Yoshimura 2000). Such models have been criticized for their lack of biological plausibility. Although this may often be true, these models may not be completely unrealistic for cases such as the explosive radiation of Lake Victoria cichlids. Conditions similar to those simulated in these models may arise if either preference and trait mutations, before being exposed to disruptive selection, accumulated in an environment in which they were selectively neutral (for example, in turbid water where colour vision is impaired), or if interspecific hybridization injects female preference and male trait genes into a population at high frequencies. Populations of *P. nyererei* and *P. pundamilia* hybridize in turbid water (Seehausen 1997; Seehausen *et al.* 1997) and the single polymorphic populations of *Pundamilia* found in very turbid water, exhibit broad variation in male nuptial colour (Seehausen *et al.* 1997) and female mating preferences (O. Seehausen, unpublished data). Similar conditions are likely to have affected other Lake Victoria cichlids in the past. In fact, against the background of far reaching climatic fluctuations within the Holocene and Late Pleistocene (Johnson *et al.* 1996, 2000), the entire Lake Victoria cichlid species flock may have gone through cycles of conditions that promote hybridization and conditions that promote speciation. Hence, it is not unlikely that the ancestral condition from which sympatric species pairs of Lake Victoria cichlids rapidly originated was one of relatively broad genetic variation in female mating preferences and male traits, as opposed to a monomorphic ancestral condition.

In speciation models that allow mating preferences to diverge or be reinforced by disruptive natural selection, the probability of sympatric speciation is less constrained by the number of genes that determine female mating preferences (Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999; Arnegard & Kondrashov 2004). The same may be true without natural selection when male nuptial colour plays a role not just in female choice, but also in male–male competition (Evans & Norris 1996; P. D. Dijkstra, O. Seehausen and T. G. G. Groothuis, unpublished data). The latter may then generate negative frequency-dependent selection on male nuptial colour, assisting the invasion of rare male phenotypes, which in turn could exert selection on initially rare female preference phenotypes (Seehausen & Schluter 2004; van Doorn *et al.* 2004; P. D. Dijkstra, O. Seehausen and T. G. G. Groothuis, unpublished data).

Although our data do not allow conclusions on the importance of disruptive natural versus disruptive sexual selection during cichlid fish speciation, they suggest that the genetics of female mating preferences in a sister species pair of Lake Victoria cichlids may be relatively simple and permissive of sympatric speciation by divergence in mate

choice through a variety of mechanisms. They further suggest that linkage mapping of female preference genes in this system might be feasible. The latter could be an important next step towards identifying the nature of female mating preferences and its role in rapid speciation.

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