

1 **The genetics of mate preferences in hybrids between two young and sympatric Lake**
2 **Victoria cichlid species**

3

4 Ola Svensson^{1,2}, Katie Woodhouse^{1,3}, Cock van Oosterhout^{1,4}, Alan Smith¹, George F
5 Turner^{1,5*}, Ole Seehausen^{6,7*}

6

7 #Corresponding author: ola.svensson@bioenv.gu.se

8 *Equal contribution

9

10 ¹ School of Biological, Biomedical and Environmental Sciences, University of Hull,
11 Hull HU67RX, UK

12 ² Present address: Department of Biological and Environmental Sciences, University of
13 Gothenburg, Box 463, Gothenburg 405 30, Sweden

14 ³ Present address: Easton and Otley College, Easton Campus, Easton, Norwich NR4 7TJ, UK

15 ⁴ Present address: School of Environmental Sciences, University of East Anglia, Norwich
16 Research Park, Norwich NR9 5DX, UK

17 ⁵ Present address: School of Biological Sciences, Bangor University, Bangor, Gwynedd LL57
18 2UW, UK

19 ⁶ Department of Fish Ecology and Evolution, EAWAG Swiss Federal Institute of Aquatic
20 Science and Technology, Center for Ecology, Evolution and Biogeochemistry, Seestrasse 79,
21 6047 Kastanienbaum, Switzerland

22 ⁷ Aquatic Ecology and Evolution, Institute of Ecology and Evolution, University of Bern,
23 Baltzerstrasse 6, 3012 Bern, Switzerland

24

25 **Abstract**

26 The genetic architecture of mate preferences are likely to affect significant evolutionary
27 processes, including speciation and hybridisation. Here, we investigate laboratory hybrids
28 between a pair of sympatric Lake Victoria cichlid fish species that appear to have recently
29 evolved from a hybrid population between in similar predecessor species. The species
30 demonstrate strong assortative mating in the lab associated with divergent male breeding
31 colouration (red dorsum vs blue). We show in a common garden experiment, using DNA-
32 based paternity testing, that the strong female mate preferences among males of the two
33 species are fully recovered in a large fraction of their F2 hybrid generation. Individual hybrid
34 females often demonstrated consistent preferences in multiple mate choice trials (≥ 5) across a
35 year or more. This result suggests that female mate preference is influenced by relatively few
36 major genes or genomic regions. These preferences were not changed by experience of a
37 successful spawning event with a male of the non-preferred species in a no-choice single-male
38 trial. We found no evidence for imprinting in the F2 hybrids, although the F1 hybrid females
39 may have been imprinted on their mothers. We discuss this nearly Mendelian inheritance of
40 consistent innate mate preferences in the context of speciation theory.

41

42 **Key words**

43 Assortative mating, hybridization, *Pundamilia nyererei*, *Pundamilia pundamilia*, sensory
44 drive, speciation-with-gene-flow

45

46 **Introduction**

47 Behavioural assortative mating is considered to play a significant role in the origin and
48 maintenance of reproductive isolation among species [1, 2]. The rate of and constraints to the
49 evolution of behavioural assortative mating is likely often influenced by the genetic
50 architecture of mate preferences and the nature and strength of genetic and non-genetic
51 influences, such as imprinting and experience. For example, modelling studies suggest that
52 sympatric and parapatric speciation starting from a monomorphic population is more probable
53 in cases where assortative mating or female preference among male courtship genotypes is
54 influenced by relatively few genetic loci [3-5], although models starting from large standing
55 variation may not have this constraint of preference architecture [6]. However, a small
56 number of preference genes tends to facilitate speciation in many models of speciation with
57 gene flow [7, 8]. Empirical studies of the genetics of species divergence in mating preferences
58 are still rare. Some of the empirical results are consistent with few genes having a major effect
59 on female assortative mating in cichlid fish and *Heliconius* butterflies [9-12]. In other
60 systems, mostly insects, female choice appears to have a more quantitative genetic
61 background [13-15].

62

63 The Lake Victoria rocky-shore cichlid fishes of the genus *Pundamilia* have emerged as a
64 significant model system for the study of speciation, being representatives of a spectacular
65 hyperdiverse, rapid adaptive radiation and being relatively tractable as a laboratory species for
66 breeding and mate choice experiments [16, 17]. Following the completion of their genome
67 sequence [18], the evolutionary history of focal populations in the SE part of the lake has been
68 reconstructed [19]. Analysis of genome-wide sequence data indicates that the species with red
69 dorsum (*P.* ‘nyererei-like’) and blue (*P.* ‘pundamilia-like’) males at Python Island have

70 recently diverged in situ, following a period of massive introgression with resident *P.*
71 *pundamilia* on the colonisation of the island by *P. nyererei* from elsewhere in the lake [19].

72

73 The *Pundamilia* species, like other haplochromine cichlid fishes, show strong sex role
74 differentiation and associated sexual dimorphism: the smaller, cryptic females are
75 mouthbrooders, caring for the offspring for several weeks, while the larger brightly coloured
76 males defend territories and display to attract females, but play no part in rearing the offspring
77 [20]. Such a breeding system is likely to generate strong sexual selection acting through
78 male-male competition and female preference for male courtship traits [21]. Closely-related
79 haplochromine species often differ markedly in male nuptial colour and it has been proposed
80 that this is associated with divergent female mate preferences [22], which have been
81 demonstrated in a number of experimental trials [23-25]. The resultant assortative mating
82 between females with a certain preference and males expressing the corresponding trait may
83 play a significant role in the maintenance and perhaps sometimes the origin of reproductive
84 isolation among sympatric species [16].

85

86 In the *Pundamilia* red/blue system, increasing water depth is associated with differentiation in
87 alleles at the long wavelength sensitive opsin gene (*LWS*), female preferences and male
88 nuptial colour, and it is likely that the sensory environment along this microhabitat gradient
89 has influenced divergence through a process of ‘sensory drive’ [26]. Of course, mating signals
90 are often multimodal and subject to multivariate selection [27-29] which is most likely also
91 the case in *Pundamilia* [16, 17, 30]. However, in the *Pundamilia* system, female preferences
92 for male nuptial colouration – itself likely to be oligogenic [31] – appear to be necessary and
93 sufficient for assortative mating [30, 32, 33].

94

95 In haplochromine cichlids, trait segregation in F2 hybrids has been shown for female
96 preferences [9, 12], male nuptial colouration [12, 31, 34] and male attractiveness to parental
97 species [33, 35]. This includes the *Pundamilia* system, where, furthermore, studies suggest an
98 absence of physical linkage between male nuptial colour and female mate preference [36]. At
99 Python Island, gene flow between the species is estimated to be ongoing [19]. Therefore, the
100 observed strong linkage disequilibrium between male colour and female preference is likely
101 to be maintained by divergent selection. A behavioural study on the second generation (F2)
102 hybrid offspring of *P. sp.* “pundamilia-like” and *P. sp.* “nyererei-like” crosses by Haesler and
103 Seehausen [9] revealed that female mate preference has a genetic basis, and that there may be
104 as few as 1 to 5 major genes that contribute to the variation in this trait. That study, however,
105 used a behavioural assay to measure mate choice, which may not be entirely predictive of
106 actual mating decisions. Here, we used a ‘common garden’ approach with full-contact
107 spawnings to examine female mate choice decisions in first and second-generation hybrids
108 (F1 and F2). Wild-type females were included as a control. We used molecular paternity
109 determination to measure directly the mating decisions of females in the laboratory [24] and
110 examined the repeatability (≥ 5 spawning decisions) of female mate choice over a year or
111 more to estimate the segregation of mate preferences in the F2 hybrids of the sympatric sister
112 species of *Pundamilia* from Python island. In contrast to Haesler and Seehausen [9], we
113 examined if mate preferences are consistently maintained across many spawning events (the
114 fully cycle from egg maturation to spawning).

115

116 If female preference is a polygenic quantitative trait with an additive genetic basis, F2 hybrids
117 preferences is expected to be distributed in a Gaussian-like fashion with few individuals
118 expressing significant preferences in the tails of the distribution. In contrast, for a polygenic
119 trait with strong dominance effects, the preference distribution of the F2 will be skewed

120 towards either end of the distribution [37-39]. On the other hand, if preferences are not
121 genetically determined, the preference distribution of F2 females is predicted to be more
122 uniform across F2 females given that individuals shared the same common environment.
123 However, in the case of gene flow, linkage disequilibrium between alleles in a polygenic trait
124 will be broken up by recombination [40, 41] and polygenic mating preferences will be
125 difficult to maintain under such conditions. Because ongoing gene flow [17, 19, 26] and
126 recombination (J. Meier and O. Seehausen, unpublished data) have been shown in this young
127 [19] species pair, and because differentiation in polygenic mating preferences will be difficult
128 to maintain under such conditions, we predicted mate preferences to segregate as an
129 oligogenic trait in a nearly Mendelian fashion.

130

131 **Methods**

132 *The experimental fish*

133 We used the sympatric sister species *Pundamilia* sp. “pundamilia-like” and *Pundamilia* sp.
134 “nyererei-like” (sensu Meier et al. [19]). These taxa show a striking difference in male nuptial
135 colours: *P.* sp. “pundamilia-like” males are grey on the flanks between black vertical bars and
136 have a metallic blue spinous dorsal fin, whereas *P.* sp. “nyererei-like” are orange on the
137 dorsum, dorsal head surface and dorsal fin and yellow on the flanks between black vertical
138 bars. It is estimated that there is currently a low to moderate level of gene flow between the
139 taxa at Python Island (The effective number of haploid immigrants per generation [$2Nm$]
140 forward in time is 0.7 from *P.* sp. “pundamilia-like” to *P.* sp. “nyererei-like” and 7.2 in the
141 opposite direction [19]). Species differences in female mate choice and divergent alleles at the
142 *LWS* opsin gene are not completely fixed [17] and males with intermediate colouration are
143 found [26]. In contrast, at Makobe Island in the open lake the sympatric species pair *P.*
144 *pundamilia* and *P. nyererei* shows stronger genome-wide differentiation, is more ecologically

145 differentiated, intermediate phenotypes are not observed and no mismatches have been
146 reported between male colouration and *LWS* opsin allele [17, 19, 26]. Both species are diploid
147 and have 22 chromosomes ($2n=44$) [18].

148

149 Wild-type females and two F1 hybrid families (one in each cross direction) used in the mate
150 choice experiment were bred from wild-caught parents. The fry were raised in stock tanks
151 until large enough to be tagged with an integrated transponder (PIT tag), to enable individual
152 identification. Using microsatellite DNA parentage analyses, we concluded that the 15 *P. sp.*
153 “pundamilia-like” females originated from 3 wild mothers and 1 wild sire and the 6 *P. sp.*
154 “nyererei-like” females from 3-6 wild mothers and 5 wild sires.

155

156 The two F2 families used in the mate choice experiment were bred from a lab stock collected
157 in 1992 [42]. The F1 families were bred from the second to third lab generation. The F2
158 generations were bred by holding one F1 male (no replacement, $N=3$) together with not more
159 than 10 F1 females in the same aquarium. One F2 half-sib family (PN1-33) was bred from
160 fish from two F1 families bred in 1999 from a female *P. sp.* “pundamilia-like” x male *P. sp.*
161 “nyererei-like”, and vice versa. This was the same F2 family used by Haesler and Seehausen
162 [9]. The F2 broods were kept separate and hence some broods in the electronic supplemental
163 material figure S3 may have had the same mother, whereas we know which of the two males
164 was the father. The other F2 family (PN34) was bred from fish from one F1 family bred in
165 2001 from a female *P. sp.* “pundamilia-like” x male *P. sp.* “nyererei-like”. The offspring were
166 pooled into the same aquaria and hence the father is known but not the brood or mother.
167 When F2 offspring were large enough, they were PIT-tagged and pooled into the same
168 aquaria. The breeding set-up is given in the electronic supplementary material, figure S1.

169

170 All females had been brooded in the mouth of their mothers until independently feeding and
171 were then raised apart from their mothers. In the data analyses we have included all spawning
172 wild type and F1 females and the 69 F2 females which spawned ≥ 5 times in the experiment.
173 Spawning decisions of females with ≤ 4 spawning decisions are given in the electronic
174 supplementary material (figure S3 and table S1) and were also used in the calculations of
175 paternal and brood effects.

176

177 *Mate choice*

178 Mate choice was tested using a “partial partition” design [24]. An aquarium measuring L 600
179 cm x W 80 cm x H 40 cm was divided into ten equally-sized compartments by plastic grids, 8
180 containing one male each, 4 of each species. Identical halved flower pots (D = 270 mm, L =
181 220 mm) served as the focal point in male territories. Two chambers were accessible to
182 females only. We used several males of each species to decrease the chance that effects of
183 individual variation in male attractiveness could override female mating preferences for males
184 of one species or the other. The mesh size of the plastic grids was adjusted to confine males in
185 their compartments, but to allow the smaller females to pass through. The complement of
186 males was replaced every second month and the female-only compartments were relocated. In
187 total, 11 wild caught and 8 lab-bred *P. sp.* “pundamilia-like” males and 11 wild caught and 6
188 laboratory-bred *P. sp.* “nyererei-like” males were used in the experiment (Electronic
189 supplementary material table S3). Wild type females were tested as a control that species-
190 specific mating preferences would be expressed in this setup. All females were tested with
191 wild type males; hybrid males were not used in these experiments.

192

193 To test whether experience altered mating preferences, 16 F2 females that had spawned 6
194 broods each and whose preferences were hence known were isolated in a tank with a male of

195 the non-preferred species. The 5 *P. sp.* “pundamilia-like”-preferring females had spawned 90-
196 100% with *P. sp.* “pundamilia-like” (mean= 98 %), and the 11 *P. sp.* “nyererei-like”-
197 preferring females had spawned 83–100% with *P. sp.* “nyererei-like” (mean= 96 %). The
198 females that subsequently spawned with a male of the species they had not preferred (N=9)
199 were allowed to brood fry until final release and potential independence of the fry. Thereafter,
200 they were released back into the experimental tank and allowed to spawn again with a choice
201 of males.

202

203 All experimental fish were marked with PIT tags and a small piece of the dorsal fin was cut to
204 provide a DNA sample. Females with eggs were placed in a separate aquarium until the eggs
205 hatched. All larvae/juveniles were euthanized using MS-222 (tricaine methanesulfonate) and
206 stored in 95% ethanol prior to paternity analyses. All females were released back into the
207 experimental tank after handling.

208

209 *Paternity analyses*

210 The experiment lasted 2.5 years. Five embryos from each brood were genotyped at 2-5
211 microsatellite loci, Ppun5, Ppun7, Pun17, Ppun21 and Ppun32. Methods for DNA extraction
212 and PCR reactions were as described previously [33] with additional optimizations for
213 multiplex analyses. The amplified DNA samples were genotyped on a Beckman Coulter CEQ
214 8000 capillary sequencer. Genotypes were received from the CEQ 8000 Series Genetic
215 Analysing System 8.0.52. Paternities were determined by direct inspection of the allele size
216 estimates on a spreadsheet, and males that possessed two alleles in a microsatellite locus that
217 were not present in the offspring were excluded as a possible father (electronic supplementary
218 material, table S1-S4). We used the number of spawning decisions in figures and statistical
219 calculations i.e. if a brood was confirmed to be fathered by more than one male each male was

220 considered to be a spawning decision. F2 females in the analysed data had 4-8 broods each
221 and 5-15 spawning decisions. The complete datasets of the wild type females, F1 hybrid
222 females, F2 hybrid females and the males used in the experiment are included in the
223 electronic supplementary material, figure S2-3 and table S1-S4. We also provide pictures of
224 the F2 males from PN1-33 in figure S4.

225

226 *Statistics*

227 When analysing between-group preferences (*P. sp.* “pundamilia-like vs. *P. sp.* “nyererei-
228 like”; F1 hybrid females with *P. sp.* “pundamilia-like” mother vs. F1 hybrid females with *P.*
229 *sp.* “nyererei-like” mother), we, for each female, subtracted the number of spawning decisions
230 with males of *P. sp.* “nyererei-like” from the number of spawning decisions with males of *P.*
231 *sp.* “pundamilia-like” and analysed the differences with Mann Whitney U-tests.

232

233 Within-group preferences were analysed with Wilcoxon signed ranks tests on the individual’s
234 number of spawning decisions with *P. sp.* “pundamilia-like” and *P. sp.* “nyererei-like”. In one
235 F1 family, a binomial test was used due to the low number of spawning decisions per female.
236 The preference of individual F2 hybrid females were also analysed with binomial tests. We
237 could not estimate individual female preferences of wild type and F1 hybrid females given the
238 small number of decisions obtained from each female.

239

240

241 To test whether the F2 female spawning patterns deviated significantly from random, we
242 simulated a distribution of spawning decisions of the 69 females that had ≥ 5 spawning
243 decisions with either a *P. sp.* “pundamilia-like” (*Pp*) or a *P. sp.* “nyererei-like” (*Pn*) male. To
244 express the level of deviation from randomness, we calculated the consistency of an

245 individual's mate choice and calculated the repeatability (R) of a female's spawning
246 decisions. In quantitative genetics, the repeatability can be used to determine the upper-bound
247 estimate of the broad sense heritability ($H^2 = V_G/V_P$) [p. 136-138, 37]. The broad sense
248 heritability indicates the relative proportion of total phenotypic variation of a trait (V_P) that
249 has a genetic basis (V_G). Repeatability is an upper-bound estimate of this heritability, given
250 that similarity in a trait value (in this case, consistent preference for males of one of the two
251 species) can both have a genetic and an environmental basis (e.g. a given female may prefer
252 males of a given species due to previous experiences). The model assesses the extent to which
253 a female's first spawning decision can predict her subsequent decisions, as this informs us on
254 how strong a mate preference has been expressed. In other words, the model assesses how
255 significantly the pattern of spawning decisions deviates from a random pattern (i.e. no
256 preference) when analysed across all F2 females at the population level. In the simulations,
257 each female is given a probability of mating with a Pp or a Pn male equivalent to the
258 proportion of P . sp. "pundamilia-like" and P . sp. "nyererei-like" spawning decisions
259 observed. This probability determines her first spawning decision. However, once a female
260 has been allocated a mate preference, the strength with which this preference continues to
261 affect subsequent spawning decisions is given by the following formulae:

262

$$P(x_i = Pp) = Pp + R(1 - Pp)$$

$$P(x_i = Pn) = Pn + R(1 - Pn)$$

263

264 Here, $P(x_i = Pp)$ and $P(x_i = Pn)$ are the probabilities of spawning with a Pp and a Pn
265 male at the i^{th} spawning decision ($i > 1$), and Pp and Pn are the observed proportions of
266 spawning decisions (across the entire population) with a P . sp. "pundamilia-like" and a P . sp.
267 "nyererei-like" male, respectively. R is the repeatability coefficient ($0 \leq R \leq 1$). With $R=0$,

268 spawning is “random” and proportional to the observed proportion of Pn and Pp spawning
269 decisions. In this case, female choice will switch randomly between Pp and Pn males. With
270 $R=1$, however, spawning choice is fixed and all spawning decisions are for males of the same
271 species as the first choice. In this case, females will consistently choose either a Pp or a Pn
272 male. With intermediate values of R , there is a preference for a species of male, but this
273 preference will not completely determine a spawning decision.

274

275 Furthermore, we also calculated if the number of individuals with preference for one species
276 differed from random. When categorizing female preference for males of either one of the two
277 species we used binomial tests and $\alpha = 0.05$ for the data set that included females with ≥ 6
278 spawning decisions.

279

280 To address potential parental and brood effects, all 100 F2 females were divided into two
281 categories: majority of spawnings with P . sp. “pundamilia-like” and majority of spawnings
282 with P . sp. “nyererei-like”. Four females were omitted because they spawned equally many
283 times with males of the two species leaving 96 females (see the electronic supplementary
284 material figure S3). We used Binomial tests to ask if the female offspring of each of the three
285 F1 fathers were biased in their spawning decisions towards one of the two species, and χ^2 to
286 test if there was a difference between F2 females fathered by different F1 males. When
287 analysing the brood effect we restricted the analyses to the 9 broods with ≥ 4 F2 females and
288 performed 36 pairwise Fisher exact test comparisons and Bonferroni correction to correct for
289 multiple comparisons.

290

291 Statistics were performed in SPSS v. 23. The individual-based model was constructed in
292 Minitab 12.1.

293

294 *Ethics*

295 This work was ethically reviewed and performed under a UK Government Home Office

296 Licence (PPL 60/3295).

297

298 **Results**

299 *Wild type females spawned with their own species*

300 There was a significant difference in spawning decisions between females of the two species

301 (Mann Whitney U test, $n = 20$, $U = 0.00$, $p < 0.001$, the electronic supplementary material

302 figure S2a). The *P. sp.* “pundamilia-like” females had 1-3 spawning decisions each (median

303 2), and 14 out of 15 spawned only with conspecific males. One female mated once with *P. sp.*

304 “nyererei-like” and twice with conspecific males (Wilcoxon signed ranks test $T = 0$, $n = 15$, p

305 < 0.001). The *P. sp.* “nyererei-like” females also had 1-3 spawning decisions each (median 3),

306 and all 6 spawned only with conspecific males (Wilcoxon signed ranks test $T = 0$, $n = 6$, $p =$

307 0.024).

308

309 *F1 hybrid females generally spawned with the species of their mother*

310 There was a significant difference in spawning decisions between the two F1 families (Mann

311 Whitney U test, $n = 16$, $U = 2.50$, $p = 0.002$, the electronic supplementary material figure

312 S2b). This was caused by F1 hybrid females spawning more often with the species of their

313 mothers (*P. sp.* “pundamilia-like” mother, 2-3 spawning decisions per female, median 2; 2

314 females spawned with both species, 9 with *P. sp.* “pundamilia-like” only, $N = 11$, Wilcoxon

315 signed ranks test, $z = 45$, $p = 0.004$, *P. sp.* “nyererei-like” mother, 1 spawning decision each,

316 all spawned with *P. sp.* “nyererei-like”, two tailed Binomial test, $n = 5$, $p = 0.063$).

317

318 *F2 hybrid spawning consistency suggests innate mating preference*

319 When including females with ≥ 6 spawning decisions and $\alpha=0.05$, 41 out of 59 F2 females had
320 a significant preference for males of one of the two species, whereas we would have expected
321 < 3 if females mated randomly (Fisher exact test, $p < 0.001$; figure 1). The simulation model
322 showed that the pattern of spawning decisions significantly deviated from a random pattern
323 when analysed at the population level. Spawning preferences segregated in an almost
324 Mendelian fashion and the majority of the females repeatedly spawned with one of the two
325 species (figure 1). The model estimates a repeatability of spawning decisions of $R=0.7$ (figure
326 2), which indicates that in our F2 population, 70% of the variation in spawning decisions is
327 explained by actual female mate preference.

328

329 To address potential parental effects, all 100 F2 females (the electronic supplementary
330 material figure S3) were divided into two categories: majority of spawnings with *P. sp.*
331 “pundamilia-like” and majority of spawnings with *P. sp.* “nyererei-like”. The female
332 offspring of the 3 F1 males were not significantly biased towards preferring either of the two
333 species (16:27, 12:16 and 11:14, Binomial tests $p=0.072$, $p=0.57$ and $p=0.69$) and there was
334 no difference in ratios between the offspring of the 3 males ($\chi^2=0.384$, $df=2$, $p=0.82$). The
335 experimental design of the present study did not allow us to confidently rule out that females
336 from different broods differed in preferences, because most broods were small. However, the
337 data rule out a general maternal effect. When restricting the analyses to broods with ≥ 4
338 females, 4 out of 36 pairwise comparisons between broods yield $p < 0.05$ with the lowest p
339 being $p=0.015$. All these are far from significant when correcting for multiple comparisons.
340 Furthermore, while their F2 brothers show considerable colour segregation within broods,
341 there is no indication of a strong correlation between a female’s preference and the colour
342 phenotype of her brothers (electronic supplementary material figure S4).

343

344 *There is no sign of copying of previous choice*

345 Only 26 out of the 69 F2 females with ≥ 5 spawning decisions spawned with both species. Of
346 those females, 21 switched back and forth between species (figure 1). This demonstrates that
347 females do not simply copy their first mate choice or their most recent choice. In other words,
348 the high repeatability of mate choice decision is unlikely to be the result of copying a previous
349 choice.

350

351 Six of the 16 F2 females with a significant mating preference, which were enclosed with a
352 male of the non-preferred species, did not spawn at all, and one female that did spawn, did not
353 spawn again when reintroduced to the large choice experiment tank. The nine females that
354 had spawned in the no-choice situation against their preference and subsequently spawned
355 again in the choice experiment, all reverted to spawning with males of the previously
356 preferred species (*P. sp.* “pundamilia-like” preferring N=3, *P. sp.* “nyererei-like” preferring
357 N=6, Two tailed Binomial test $p = 0.004$) which highlights the innate strength of female mate
358 preference.

359

360 **Discussion**

361 The genetics of female mate preferences is likely to affect evolutionary processes, including
362 speciation and hybridisation between species. We report a long term common garden study
363 where we followed spawning decisions of F2 hybrid females between two sympatric sister
364 species throughout a large part of their reproductive lives as well as spawning decisions of F1
365 hybrid females. Wild type females of both species were used as a control.

366

367 Using molecular paternity testing, our experiments indicated that wild-type females mostly
368 mated with conspecific males, although mating was not 100% assortative. This is consistent
369 with the results of previous studies on the same population using mating experiments [30] or
370 behavioural preference assays [9, 30, 42, 43], and indicates that either method can be used
371 reliably to estimate preferences. The occasional disassortative mating is also consistent with
372 modelling based on population genomic data suggesting ongoing gene flow between the same
373 sympatric species in nature, as well as between allopatric populations [19].

374

375 All F1 hybrid females mated with their maternal species, although a couple of them also
376 mated with the paternal species. This bias towards the maternal species is consistent with an
377 effect of imprinting, which had previously been demonstrated in Lake Victoria
378 haplochromines using controlled cross-fostering experiments with mate preferences assayed
379 with a behavioural choice test [44, 45]. Our results are, however, also consistent with the
380 possibility that genes influencing species-specific preferences were not entirely reciprocally
381 fixed between the wild-type individuals used to breed our F1 hybrids, e.g. as a result of
382 occasional introgression [19, 26]. It is not impossible that one of the parents of our two test F1
383 families may have been heterozygous at a mate preference locus, and that thus some of the F1
384 hybrid females were homozygous.

385

386 By contrast, the experimental design limited the potential for any imprinting of species-
387 specific preferences in F2 hybrids, since their mothers were all F1 hybrids. Furthermore, we
388 found that siblings in most families exhibited consistent preferences for males of different
389 species, which is inconsistent with imprinting. Likewise, our experimental test of the
390 preferences of females following a 'no-choice' mating with the non-preferred male species
391 indicated that females retained their original preferences in a subsequent free choice

392 experiment, suggesting that experience did not disrupt their innate preferences. In general,
393 many F2 hybrid females were consistent in choosing males of a particular species, with 41 out
394 of 59 females showing a significant preference, far more than the 3 expected if females had
395 mated by chance. This clear nearly Mendelian segregation in spawning preferences in the F2
396 generation is consistent with previous behavioural choice tests by Haesler and Seehausen [9].
397 The Mendelian segregation despite incomplete genetic isolation [17, 19, 26] and
398 recombination (J. Meier and O. Seehausen, unpublished data) in this species pair in the wild
399 implies that species-specific female mate choice among the *Pundamilia* sister species is
400 influenced by relatively few major genes or genomic regions containing several tightly linked
401 loci.

402

403 **Repeatability and the heritability of mate choice**

404 Our simulation indicated that the distribution of spawning decisions over F2 hybrid females
405 deviated significantly from expectations if mating was random when analysed at the
406 population level. A large excess of females showed a significant preference for males of either
407 one of the two species. Female choice of certain type of males within a species often has low
408 repeatability and is subject to change depending on e.g. experience, age, condition, mate
409 copying and the environment [46-48]. In our experiment, repeatability of spawning decisions
410 of F2 hybrid females was high (70 %) and preferences did not change over time and over
411 successive reproductive cycles of females, nor after the experience of a successful spawning
412 event with a male of the non-preferred species. Repeatability is also often used to determine
413 the upper-bound estimate of the broad sense heritability (H^2) in behavioural studies [46, 47].
414 The results from our simulation therefore suggest that up to 70% of the variation in spawning
415 decisions observed among the F2 hybrid females may have a heritable basis. However, the
416 remaining 30% could simply be due to lack of a consistent preference in the class of

417 preference heterozygote F2 hybrid females – these are expected to mate randomly [9].
418 Therefore, heritability may be higher than the estimated 70% [9, 49]. In the experimental
419 design, we aimed to minimize environmental variation introduced by differences in condition
420 between males by providing a choice among eight males, four of each species in each trial.
421 Differences in territory quality were unlikely in the standardised conditions of our experiment.
422 Thus, we conclude that the observed among-female variation in preference is likely to be due
423 to genetic factors.

424

425 **Sexual isolation by mate choice**

426 Behavioural reproductive isolation is of key importance to understanding the rapid evolution
427 of genetically differentiated sympatric species [1, 41, 50], such as those in African cichlid fish
428 radiations. The species pair that we studied here has been estimated to have arisen in just
429 slightly more than 150 generations, facilitated by hybridisation between the local *P.*
430 *pundamilia* and migrants of *P. nyererei* from around Makobe island [19].

431

432 The male trait (red dorsum vs. blue colour) that species-assortative female mating preferences
433 are based on [30] is likely oligogenic itself [31]. Theoretical work suggests that it is easier for
434 divergent selection to overcome homogenizing gene flow if traits under divergent selection
435 are due to relatively few genes, because the fewer genes that are responsible for a trait under
436 divergent selection, the higher are the selection coefficients for each locus [51-53]. Hence, the
437 genetic architecture of mate choice and mating traits in *Pundamilia* may facilitate speciation
438 in the face of gene flow, perhaps in combination with other selection pressures, as might be
439 generated by adaptation to divergent microhabitats, particularly water depths: field studies
440 have shown that red dorsum males tend to be found in deeper water than the blue males [26].

441

442 **Candidate genes for mate choice**

443 Candidate genes relating to species-specific mate preferences are likely to include those
444 affecting vision. Divergence has been shown in the long wavelength sensitive opsin gene
445 (*LWS*) [26]. In the red vs. blue species pair at Makobe Island, there is also divergence in the
446 short wavelength sensitive opsin gene (*SWS2A*) but this is not currently known in the species
447 pair of the present study [26]. At Makobe Island, there is also divergence in other putative
448 coding regions [18], some of which may be related to vision.

449

450 Many small genomic ‘islands of differentiation’ were found to differentiate *P. pundamilia* and
451 *P. nyererei* from Makobe Island [18]. However, the Python Island species pair having
452 recently (around 150 generations ago) re-emerged after a period of massive introgression
453 might be expected to be divergent at fewer regions, more directly related to divergent
454 selection pressures, which should make traits directly related to reproductive isolation easier
455 to detect. Malinsky et al. [54] identified several genomic regions with high differentiation in
456 two young ecomorphs of crater lake haplochromine cichlids (genus *Astatotilapia*) with partial
457 assortative mating. Candidate adaptive genes in these so called ‘genomic islands of
458 differentiation’ included rhodopsin and other twilight-vision-associated genes. Differentiation
459 in such ‘islands’ can resist ongoing gene flow, as shown in < 150 year old incipient
460 *Gasterosteus* stickleback species pairs in two Swiss lakes [55, 56].

461

462 **To conclude**

463 We show in a common garden long term mating experiment that strong female mating
464 preferences for males of either one of two sister species are recovered in large fractions of the
465 F2 hybrid generation. The genetic assays of mate choice in F2 hybrids between *P. sp.*
466 “pundamilia-like” and *P. sp.* “nyererei-like” show high repeatability and consistency in

467 female choice across many reproductive cycles, and we argue that the variation is influenced
468 by the segregation of a few genes with large effects. We propose that a simple genetic basis
469 could help facilitate stable phenotypic differentiation in sympatry in the face of gene flow.

470

471 **Data accessibility**

472 The complete datasets of the wild type, F1 and F2 females, and the males used in the
473 experiment are included in figure 1 and the electronic supplementary material, figure S2-3
474 and table S1-S4. The raw data in the electronic supplementary material, table S1-S4 are also
475 available from the Dryad Digital Repository <http://dx.doi.org/10.5061/dryad.q58hr> [57]. The
476 Minitab 12.1 macro to test the repeatability of mate choice is deposited at GitHub
477 <https://github.com/Ward9250/FishSpawn>

478

479

480 **Authors' contribution**

481 G.F.T. and O.Se. conceived the project, O.Sv., G.F.T. and O.Se. designed the experiments,
482 K.W. and A.S. carried out the crosses, O.Sv., K.W. and A.S. performed the experiments,
483 O.Sv. carried out microsatellite paternity analyses and processed the data, C.v.O. wrote the
484 simulation model, O.Sv. and C.v.O. analysed the data, O.Sv. wrote the manuscript with
485 important contribution from C.v.O, G.F.T. and O.Se. All authors have provided critical
486 revision of the manuscript and approved the final version.

487

488 **Competing interest**

489 We declare no competing interest

490

491 **Funding**

492 This project was funded by a BBSRC Standard Grant G20313 (to G.F.T and O.Se).
493 Additional support (to O.Sv.) were provided by University of Gothenburg and the Linnaeus
494 Centre for Marine Evolutionary Biology at the University of Gothenburg
495 (<http://www.cemeb.science.gu.se>), and to C.v.O. by the Earth and Life Systems Alliance
496 (ELSA), Norwich Research Park, UK.

497

498 **Acknowledgements**

499 We are grateful for the essential help in the aquarium facility and genetic laboratory from Bill
500 Hutchinson, Domino Joyce, Paul Nichols, Michele Pierotti, Noel Wreathall and Helen
501 Wilcock. Joana Meier commented on the manuscript. The manuscript was also greatly
502 improved by the helpful comments from the editor and two anonymous reviewers.

503

504 **References**

505

- 506 1. Coyne J.A., Orr H.A. 2004 *Speciation*. Sunderland, Sinauer.
- 507 2. Nosil P. 2012 *Ecological Speciation*. Oxford, Oxford University Press; 280 p.
- 508 3. Arnegard M.E., Kondrashov A.S. 2004 Sympatric speciation by sexual selection
509 alone is unlikely. *Evolution* **58**, 222-237. (doi:10.1111/j.0014-3820.2004.tb01640.x).
- 510 4. Doebeli M. 2005 Adaptive speciation when assortative mating is based on
511 female preference for male marker traits. *J Evol Biol* **18**, 1587-1600. (doi:10.1111/j.1420-
512 9101.2005.00897.x).
- 513 5. Lande R. 1981 Models of speciation by sexual selection on polygenic traits.
514 *Proc Natl Acad Sci USA* **78**, 3721-3725. (doi:10.1073/pnas.78.6.3721).
- 515 6. Higashi M., Takimoto G., Yamamura N. 1999 Sympatric speciation by sexual
516 selection. *Nature* **402**, 523-526. (doi:10.1038/990087).

- 517 7. Kawata M., Shoji A., Kawamura S., Seehausen O. 2007 A genetically explicit
518 model of speciation by sensory drive within a continuous population in aquatic environments.
519 *BMC Evol Biol* **7**, 99. (doi:10.1186/1471-2148-7-99).
- 520 8. Thibert-Plante X., Gavrilets S. 2013 Evolution of mate choice and the so-called
521 magic traits in ecological speciation. *Ecol Lett* **16**, 1004-1013. (doi:10.1111/ele.12131).
- 522 9. Haesler M.P., Seehausen O. 2005 Inheritance of female mating preference in a
523 sympatric sibling species pair of Lake Victoria cichlids: implications for speciation. *Proc R*
524 *Soc B* **272**, 237-245.
- 525 10. Kronforst M.R., Young L.G., Kapan D.D., McNeely C., O'Neill R.J., Gilbert
526 L.E. 2006 Linkage of butterfly mate preference and wing color preference cue at the genomic
527 location of wingless. *Proc Natl Acad Sci USA* **103**, 6575-6580.
528 (doi:10.1073/pnas.0509685103).
- 529 11. Merrill R.M., Van Schooten B., Scott J.A., Jiggins C.D. 2011 Pervasive genetic
530 associations between traits causing reproductive isolation in *Heliconius* butterflies. *Proc R*
531 *Soc Lond, Ser B: Biol Sci* **278**, 511-518. (doi:10.1098/rspb.2010.1493).
- 532 12. Ding B., Daugherty D.W., Husemann M., Chen M., Howe A.E., Danley P.D.
533 2014 Quantitative genetic analyses of male color pattern and female mate choice in a pair of
534 cichlid fishes of Lake Malawi, East Africa. *Plos One* **9**. (doi:e114798
535 10.1371/journal.pone.0114798).
- 536 13. Diao W.W., Mousset M., Horsburgh G.J., Vermeulen C.J., Johannes F., van de
537 Zande L., Ritchie M.G., Schmitt T., Beukeboom L.W. 2016 Quantitative trait locus analysis
538 of mating behavior and male sex pheromones in *Nasonia* wasps. *G3-Genes Genomes Genet* **6**,
539 1549-1562. (doi:10.1534/g3.116.029074).

- 540 14. Laturney M., Moehring A.J. 2012 Fine-scale genetic analysis of species-specific
541 female preference in *Drosophila simulans*. *J Evol Biol* **25**, 1718-1731. (doi:10.1111/j.1420-
542 9101.2012.02550.x).
- 543 15. Oh K.P., Fergus D.J., Grace J.L., Shaw K.L. 2012 Interspecific genetics of
544 speciation phenotypes: song and preference coevolution in Hawaiian crickets. *J Evol Biol* **25**,
545 1500-1512. (doi:10.1111/j.1420-9101.2012.02531.x).
- 546 16. Maan M.E., Seehausen O. 2010 Mechanisms of species divergence through
547 visual adaptation and sexual selection: Perspectives from a cichlid model system. *Curr Zool*
548 **56**, 285-299.
- 549 17. Seehausen O. 2009 Progressive levels of trait divergence along a 'speciation
550 transect' in the Lake Victoria cichlid fish *Pundamilia*. In *Ecological Reviews: Speciation and*
551 *Patterns of Diversity* (eds. Butlin R., Bridle J., Schluter D.), pp. 155-176. Cambridge,
552 Cambridge University Press.
- 553 18. Brawand D., Wagner C.E., Li Y.I., Malinsky M., Keller I., Fan S., Simakov O.,
554 Ng A.Y., Lim Z.W., Bezault E., et al. 2014 The genomic substrate for adaptive radiation in
555 African cichlid fish. *Nature* **513**, 375-381. (doi:10.1038/nature13726).
- 556 19. Meier J.I., Sousa V.C., Marques D.A., Selz O.M., Excoffier L., Seehausen O.
557 2016 Demographic modeling of whole genome data reveals parallel origin of similar
558 *Pundamilia* cichlid species after hybridization. *Mol Ecol* **Epub ahead of print**.
559 (doi:10.1111/mec.13838. Epub ahead of print).
- 560 20. Seehausen O. 1996 *Lake Victoria rock cichlids - taxonomy, ecology and*
561 *distribution*. Zevenhuizen, Verduijn Cichlids; 304 p.
- 562 21. Andersson M. 1994 *Sexual selection*. Princeton, New Jersey, Princeton
563 University Press.

- 564 22. Seehausen O. 2006 African cichlid fish: a model system in adaptive radiation
565 research. *Proc R Soc Lond, Ser B: Biol Sci* **273**, 1987-1998. (doi:10.1098/rspb.2006.3539).
- 566 23. Selz O.M., Thommen R., Pierotti M.E.R., Anaya-Rojas J.M., Seehausen O.
567 2016 Differences in male coloration are predicted by divergent sexual selection between
568 populations of a cichlid fish. *Proc R Soc Lond, Ser B: Biol Sci* **283**.
569 (doi:10.1098/rspb.2016.0172).
- 570 24. Knight M.E., Turner G.F. 2004 Laboratory mating trials indicate incipient
571 speciation by sexual selection among populations of the cichlid fish *Pseudotropheus zebra*
572 from Lake Malawi. *Proc R Soc B* **271**, 675-680. (doi:10.1098/rspb.2003.2639).
- 573 25. Kidd M.R., Danley P.D., Kocher T.D. 2006 A direct assay of female choice in
574 cichlids: all the eggs in one basket. *J Fish Biol* **68**, 373-384. (doi:10.1111/j.1095-
575 8649.2005.00896.x).
- 576 26. Seehausen O., Terai Y., Magalhaes I.S., Carleton K.L., Mrosso H.D.J., Miyagi
577 R., van der Sluijs I., Schneider M.V., Maan M.E., Tachida H., et al. 2008 Speciation through
578 sensory drive in cichlid fish. *Nature* **455**, 620-U623. (doi:10.1038/nature07285).
- 579 27. Blows M.W., Brooks R., Kraft P.G. 2003 Exploring complex fitness surfaces:
580 Multiple ornamentation and polymorphism in male guppies. *Evolution* **57**, 1622-1630.
- 581 28. Brooks R., Hunt J., Blows M.W., Smith M.J., Bussiere L.F., Jennions M.D.
582 2005 Experimental evidence for multivariate stabilizing sexual selection. *Evolution* **59**, 871-
583 880. (doi:10.1111/j.0014-3820.2005.tb01760.x).
- 584 29. Candolin U. 2003 The use of multiple cues in mate choice. *Biol Rev* **78**, 575-
585 595.
- 586 30. Selz O.M., Pierotti M.E.R., Maan M.E., Schmid C., Seehausen O. 2014 Female
587 preference for male color is necessary and sufficient for assortative mating in 2 cichlid sister
588 species *Behav Ecol* **25**, 612-626. (doi:10.1093/beheco/aru024).

- 589 31. Magalhães I.S., Seehausen O. 2010 Genetics of male nuptial colour divergence
590 between sympatric sister species of a Lake Victoria cichlid fish. *J Evol Biol* **23**, 914-924.
- 591 32. Seehausen O., vanAlphen J.J.M., Witte F. 1997 Cichlid fish diversity threatened
592 by eutrophication that curbs sexual selection. *Science* **277**, 1808-1811.
- 593 33. Stelkens R.B., Pierotti M.E.R., Joyce D.A., Smith A.M., van der Sluijs I.,
594 Seehausen O. 2008 Disruptive sexual selection on male nuptial coloration in an experimental
595 hybrid population of cichlid fish. *Philos Trans R Soc Lond, B, Biol Sci* **363**, 2861-2870.
596 (doi:10.1098/rstb.2008.0049).
- 597 34. Barson N.J., Knight M.E., Turner G.F. 2007 The genetic architecture of male
598 colour differences between a sympatric Lake Malawi cichlid species pair. *J Evol Biol* **20**, 45-
599 53. (doi:10.1111/j.1420-9101.2006.01228.x).
- 600 35. Svensson O., Egger B., Gricar B., Woodhouse K., van Oosterhout C.,
601 Salzburger W., Seehausen O., Turner G. 2011 Segregation of species-specific male
602 attractiveness in F2 hybrid Lake Malawi cichlid fish. *Int J Evol Biol* **2011**, 1-7.
603 (doi:10.4061/2011/426179).
- 604 36. van der Sluijs I., Dijkstra P.D., Lindeyer C.M., Visser B., Smith A.M.,
605 Groothuis T.G.G., van Alphen J.J.M., Seehausen O. 2013 A test of genetic association among
606 male nuptial coloration, female mating preference, and male aggression bias within a
607 polymorphic population of cichlid fish. *Curr Zool* **59**, 221-229.
- 608 37. Falconer D.S., Mackay T.F.C. 1996 *Introduction to Quantitative Genetics*. 4 ed.
609 Harlow, Essex, UK, Pearson Education Limited; 480 p.
- 610 38. Lande R. 1981 The minimum number of genes contributing to quantitative
611 variation between and within populations. *Genetics* **99**, 541-553.
- 612 39. Turelli M., Barton N.H. 1994 Genetic and statistical analyses of strong selection
613 on polygenic traits: what, me normal? *Genetics* **138**, 913-941.

- 614 40. Felsenstein J. 1981 Skepticism towards Santa Rosalia, or why are there so few
615 kinds of animals? *Evolution* **35**, 124-138. (doi:10.2307/2407946).
- 616 41. Smadja C.M., Butlin R.K. 2011 A framework for comparing processes of
617 speciation in the presence of gene flow. *Mol Ecol* **20**, 5123-5140. (doi:10.1111/j.1365-
618 294X.2011.05350.x).
- 619 42. Seehausen O., van Alphen J.J.M. 1998 The effect of male coloration on female
620 mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex).
621 *Behav Ecol Sociobiol* **42**, 1-8.
- 622 43. van der Sluijs I., Van Dooren T.J.M., Hofker K.D., van Alphen J.J.M., Stelkens
623 R.B., Seehausen O. 2008 Female mating preference functions predict sexual selection against
624 hybrids between sibling species of cichlid fish. *Philos Trans R Soc Lond, Ser B: Biol Sci* **363**,
625 2871-2877. (doi:10.1098/rstb.2008.0045).
- 626 44. Verzijden M.N., Korthof R.E.M., ten Cate C. 2008 Females learn from mothers
627 and males learn from others. The effect of mother and siblings on the development of female
628 mate preferences and male aggression biases in Lake Victoria cichlids, genus *Mbipia*. *Behav*
629 *Ecol Sociobiol* **62**, 1359-1368. (doi:10.1007/s00265-008-0564-x).
- 630 45. Verzijden M.N., ten Cate C. 2007 Early learning influences species assortative
631 mating preferences in Lake Victoria cichlid fish. *Biol Lett* **3**, 134-136.
- 632 46. Jennions M.D., Petrie M. 1997 Variation in mate choice and mating preferences:
633 A review of causes and consequences. *Biol Rev* **72**, 283-327.
634 (doi:10.1017/s0006323196005014).
- 635 47. Bell A.M., Hankison S.J., Laskowski K.L. 2009 The repeatability of behaviour:
636 a meta-analysis. *Animal Behaviour* **77**, 771-783. (doi:10.1016/j.anbehav.2008.12.022).
- 637 48. Brooks R. 1996 Copying and the repeatability of mate choice. *Behav Ecol*
638 *Sociobiol* **39**, 323-329. (doi:10.1007/s002650050296).

- 639 49. Dohm M.R. 2002 Repeatability estimates do not always set an upper limit to
640 heritability. *Funct Ecol* **16**, 273-280.
- 641 50. Servedio M.R. 2016 Geography, assortative mating, and the effects of sexual
642 selection on speciation with gene flow. *Evolutionary Applications* **9**, 91-102.
643 (doi:10.1111/eva.12296).
- 644 51. Gavrillets S., Vose A. 2005 Dynamic patterns of adaptive radiation. *Proc Natl*
645 *Acad Sci USA* **102**, 18040-18045. (doi:10.1073/pnas.0506330102).
- 646 52. Yeaman S., Otto S.P. 2011 Establishment and maintenance of adaptive genetic
647 divergence under migration, selection, and drift. *Evolution* **65**, 2123-2129.
648 (doi:10.1111/j.1558-5646.2011.01277.x).
- 649 53. Yeaman S., Whitlock M.C. 2011 The genetic architecture of adaptation under
650 migration-selection balance. *Evolution* **65**, 1897-1911. (doi:10.1111/j.1558-
651 5646.2011.01269.x).
- 652 54. Malinsky M., Challis R.J., Tyers A.M., Schiffels S., Terai Y., Ngatunga B.P.,
653 Miska E.A., Durbin R., Genner M.J., Turner G.F. 2015 Genomic islands of speciation
654 separate cichlid ecomorphs in an East African crater lake. *Science* **350**, 1493-1498.
655 (doi:10.1126/science.aac9927).
- 656 55. Marques D.A., Lucek K., Meier J.I., Mwaiko S., Wagner C.E., Excoffier L.,
657 Seehausen O. 2016 Genomics of rapid incipient speciation in sympatric threespine
658 stickleback. *PLoS Genetics* **12**, e1005887. (doi:10.1371/journal.pgen.1005887).
- 659 56. Marques D.A., Lucek K., Haesler M.P., Feller A.F., Meier J.I., Wagner C.E.,
660 Excoffier L., Seehausen O. 2016 Genomic landscape of early ecological speciation initiated
661 by selection on nuptial colour. *Mol Ecol* **Epub ahead of print**. (doi:10.1111/mec.13774).
- 662 57. Svensson O., Woodhouse K., van Oosterhout C., Smith A., Turner G.F.,
663 Seehausen O. 2017 The genetics of mate preferences in hybrids between two young and

664 sympatric Lake Victoria cichlid species. *Dryad Digital Repository*.

665 (doi:doi:10.5061/dryad.q58hr).

666

667

668

669 **Figure legends**

670

671

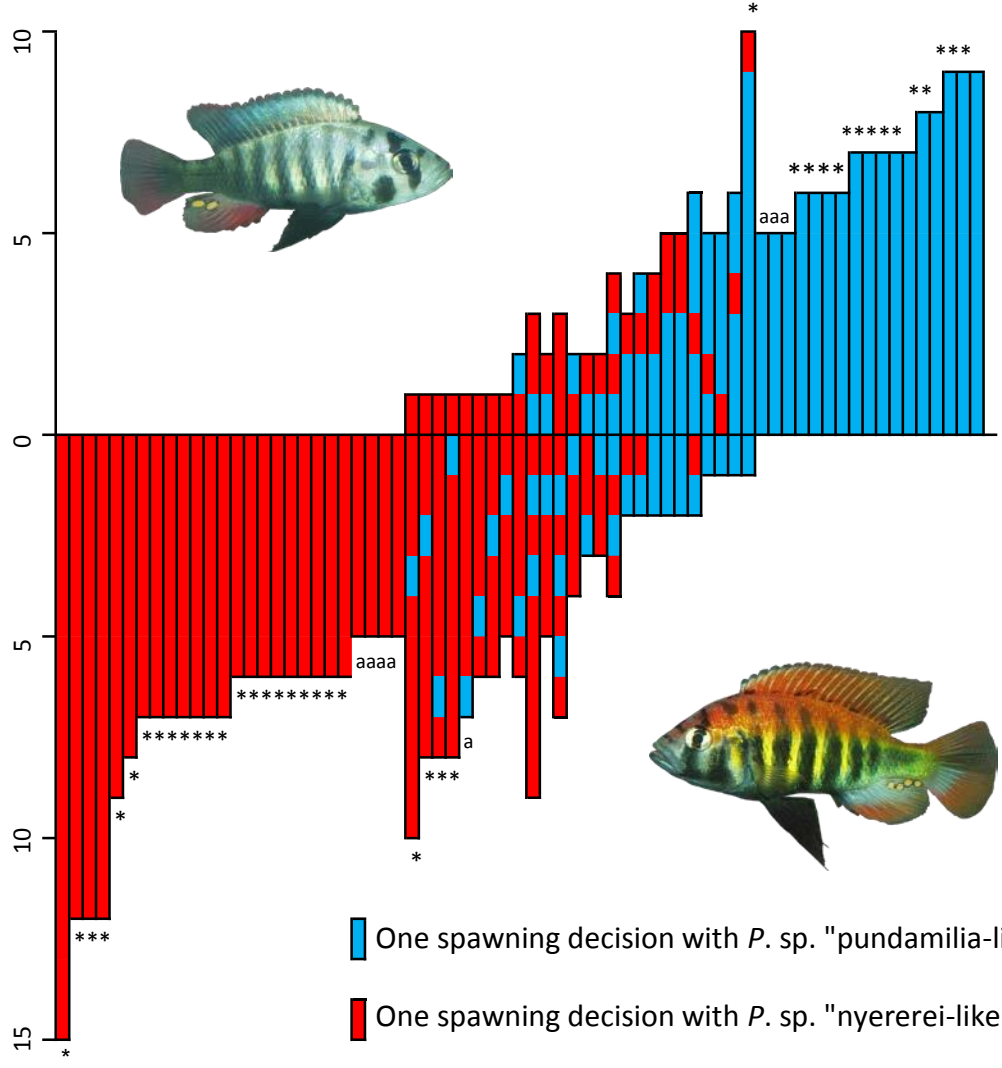
672 **Figure 1.** Individual spawning decisions by the 69 F2 hybrid females. Spawning decisions
673 were determined by microsatellite DNA paternity analyses. Above the line $y=0$ is the number
674 of spawning decisions with *P. sp.* “pundamilia-like”, and below the line is the number of
675 spawning decisions with *P. sp.* “nyererei-like”. The order of spawning decisions is arranged
676 with the first spawning on the top, and the last on the bottom with a spawning decision with
677 *P. sp.* “pundamilia-like”, marked in blue and a spawning decision with *P. sp.* “nyererei-like”
678 marked in red. * $p<0.05$, ^a $0.05<p<0.1$.

679

680

681 **Figure 2.** (A) Simulated (means and 5-95% error bars) spawning decisions of F2 females with
682 *P. sp.* “pundamilia-like” (blue dots), and with *P. sp.* “nyererei-like” (red dots) based on a
683 repeatability of an individual’s spawning decision of $R=0.7$. Observed ratio of spawning
684 decisions is shown by the solid black lines. (B) The best fit of the model is with $R=0.7$, which
685 minimises the mean squares (MS) between the observed and simulated spawning distribution.
686 Lower values of R produce a more random spawning pattern, whilst higher values of R
687 increase the consistency of a females’ spawning choices above those observed, which reduced
688 the fit of the model by inflating the MS.

Number of spawning decisions
with *P. sp. "nyererei-like"*



Order of spawning decisions



One spawning decision with *P. sp. "pundamilia-like"*
One spawning decision with *P. sp. "nyererei-like"*

