Genetic architecture of a key reproductive isolation trait differs between sympatric and non-sympatric sister species of Lake Victoria cichlids

Anna F. Feller1,3, Marcel P. Haesler1,3, Catherine L. Peichel2 and Ole Seehausen1,3

1Division of Aquatic Ecology and Evolution, Institute of Ecology and Evolution, and 2Division of Evolutionary Ecology, Institute of Ecology and Evolution, University of Bern, 3012 Bern, Switzerland
3Department of Fish Ecology and Evolution, Centre of Ecology, Evolution and Biogeochemistry, EAWAG Swiss Federal Institute of Aquatic Science and Technology, 6047 Kastanienbaum, Switzerland

One hallmark of the East African cichlid radiations is the rapid evolution of reproductive isolation that is robust to full sympatry of many closely related species. Theory predicts that species persistence and speciation in sympathy with gene flow are facilitated if loci of large effect or physical linkage (or pleiotropy) underlie traits involved in reproductive isolation. Here, we investigate the genetic architecture of a key trait involved in behavioural isolation, male nuptial coloration, by crossing two sister species pairs of Lake Victoria cichlids of the genus *Pundamilia* and mapping nuptial coloration in the F2 hybrids. One is a young sympatric species pair, representative of an axis of colour motif differentiation, red-dorsum versus blue, that is highly recurrent in closely related sympatric species. The other is a species pair representative of colour motifs, red-chest versus blue, that are common in allopatric but uncommon in sympatric closely related species. We find significant quantitative trait loci (QTLs) with moderate to large effects (some overlapping) for red and yellow in the sympatric red-dorsum × blue cross, whereas we find no significant QTLs in the non-sympatric red-chest × blue cross. These findings are consistent with theory predicting that large effect loci or linkage/pleiotropy underlying mating trait differentiation could facilitate speciation and species persistence with gene flow in sympathy.

1. Background

The adaptive radiation of Lake Victoria haplochromine cichlids comprises approximately 500 endemic species that have evolved within the lake in perhaps as little as 15 000 years [1–4] and that are highly diverse in morphology, ecology, colour and behaviour [5–7]. Typically, however, closely related species are similar in morphology and ecology while they differ dramatically in male nuptial coloration [6,8,9]. Male nuptial coloration is considered a trait of key importance in the origin and maintenance of new species in these fish [9–11].

A highly recurrent pattern in male nuptial colour variation in pairs of closely related species of Lake Victoria cichlids is that males in one species are blue-grey on their body with any red colour confined to the fins, whereas males of the other species are yellow-red on the body [9–12]. The red colour can be confined to either dorsal parts of the body (‘red-dorsum’ type) or to the chest and lower head (‘red-chest’ type). When closely related species are sympatric, the red form generally has a red-dorsum, whereas many non-sympatric pairs of closely related species involve a red-chest and a blue form [6,8,13].

A representative case of sympatric red-dorsum and blue sister species is the young species pair of *Pundamilia* sp. ‘nyererei-like’ and *Pundamilia* sp.
Pundamilia-like at Python Island (figure 1). This pair may have evolved in sympatry from a hybrid population in only a few hundred generations [15,16,18]. Pundamilia sp. ‘red-head’ and Pundamilia pundamilia are representatives of a red-chest and blue species with overlapping geographical distributions that are never found together on the same rocky island, despite islands occupied by the different species lying within dispersal distance of each other (figure 1) [6,8,19].

Male nuptial colour in Pundamilia is under intra- and intersexual selection. Males fiercely compete for territories, and they use their bright colours to signal territory ownership to contestants. Negative frequency-dependent selection
generated by own-type aggression biases is likely involved in stabilizing the coexistence of different colour types [11,20–22]. Females, which are cryptically coloured and the sole investors into parental care, exhibit strong preferences for mates with particular nuptial coloration, which generates both directional sexual selection within and assortative mating between species [23–26]. Preference for male colour has been shown to be heritable and likely determined by few major genes or genomic regions [27,28] and to generate disruptive selection on male colour [29].

Despite strong preferences for bright male colour and assortative mating, there is some gene flow in fully sympatric red-dorsum versus blue pairs such as *Pundamilia* sp. ‘nyererei-like’ and *Pundamatila* sp. ‘pundamilia-like’ [15,16]. Nonetheless these two species persist in sympathy. With a genome-wide mean FST of 0.053 and hundreds of highly differentiated genomic regions, they show surprisingly strong genetic differentiation considering they have likely evolved in full sympatry in less than 200 generations [15,16]. Red-chest versus blue pairs like *Pundamila* sp. ‘red-head’ and *Pundamila pundamila* experience little gene flow because they usually do not co-occur. Although they are neither strictly geographically isolated nor dispersal limited [6,8,19] (and figure 1), they do not seem to persist as two species in the same island.

Here we ask whether differences in the genetic architecture of the male nuptial colour motifs that differentiate these species could explain the difference in the distribution patterns of the species pairs. We tested the hypothesis that red-dorsum versus blue pairs persisting in sympathy despite some gene flow have an architecture that is robust against potentially homogenizing effects of gene flow, while the absence of such an architecture would make it difficult for red-chest versus blue pairs to persist in the presence of gene flow.

In a sympatric scenario with ongoing gene flow, recombination is expected to break up linkage disequilibrium between favourable combinations of alleles for local adaptation and reproductive isolation [30,31]. This is less likely to occur if divergently selected traits are coded by few genes with large effects, as this reduces the number of targets for recombination to break up, and increases the effectiveness of (correlational) selection because it is concentrated on fewer targets [32]. Indeed, theoretical work has shown that large effect alleles, or groups of tightly linked alleles with smaller effects that then act like a large effect locus, are less likely to be lost when adaptive multilocus phenotypes need to be maintained against gene flow [33–35]. Similarly, the physical linkage of multiple traits (or pleiotropy) could facilitate divergence with gene flow [34,36,37].

Recombinant males between cichlid (*Pundamilia*) species with either red or blue male nuptial coloration might have reduced fitness if they will be less likely to be chosen by females with strong preferences for either colour, as is the case in these species [23–28], or if intermediate coloration makes them targets of territorial aggression by males of both species. We thus predict large effect loci and/or physical linkage of several loci (or pleiotropy) for male nuptial colour to be present in sympatric red-dorsum versus blue cichlid pairs, as such an architecture could both facilitate the establishment of polymorphisms [38] and make it easier to retain phenotypic differentiation in sympathy with ongoing gene flow (see above). Furthermore, theoretical models that investigate the feasibility of sympatric speciation by sexual selection (alone) usually find such speciation feasible when assuming a simple genetic architecture (i.e. few additive loci with large effects) and that speciation becomes less likely when the number of loci underpinning reproductive isolation traits increases [39–41].

To compare the genetic architecture of red and yellow versus blue male nuptial colour motifs that do or do not persist with gene flow, we crossed *Pundamilia* sp. ‘nyererei-like’ and *Pundamila* sp. ‘pundamila-like’ (red-dorsum × blue) and *Pundamila sp. ‘red-head’ and *Pundamila pundamila* (red-chest × blue) in the laboratory and performed quantitative trait locus (QTL) mapping analyses on male nuptial coloration in the second generation (F2) hybrids.

We find several significant QTLs for the presence/absence of red and yellow colour in the sympatric red-dorsum × blue cross, with several traits mapping to the same region, whereas we find no significant QTLs in the non-sympatric red-chest × blue cross. We conclude that the presence of large effect loci, with physical linkage between some traits (or pleiotropy), likely makes up one key element for the rapid evolution of reproductive isolation and species persistence in sympathy despite some gene flow.

## 2. Material and methods

### (a) Experimental crosses

The red-dorsum × blue cross was started with a *Pundamilia* sp. ‘nyererei-like’ female and a *Pundamilia* sp. ‘pundamila-like’ male, both from laboratory bred strains established from fishes caught by OS at Python Island in Lake Victoria in 2003 (figure 1). The red-chest × blue cross was started with a *Pundamilia pundamila* female caught by O.S. at Makobe Island in 2003 and a *Pundamila* sp. ‘red-head’ male from a laboratory bred strain established from fishes caught by O.S. at Zue Island in 1993 (figure 1). Five to 6 days after spawning, the eggs were removed from the female’s mouth and reared in an egg tumbler until hatching. After yolk sac resorption, the larvae were transferred to rearing aquaria that were part of a large recirculation system. After sexual maturity at the age of 1 to 2 years, two pairs of F1 individuals of each cross were then allowed to mate, and the eggs and larvae were reared the same way as the F1 generation. Because the average clutch size is just about 20–30 juveniles, we re-mated each F1 pair multiple times until we had obtained a total of approximately 300 F2 individuals in each cross. This procedure of repeatedly re-mating the same pairs took about 2 years. All F2s were reared to an age of at least 1 year before they were phenotyped. All four populations from which the grandparents were taken breed true in the laboratory in a common garden environment, hence the differences in coloration are heritable in both crosses.

### (b) Colour photos and scoring of coloration

Sexually mature F2 males were removed from their holding tank and individually placed into one of six adjacent plexiglass photo cuvette compartments with transparent separations and a grey PVC background, inside a larger aquarium. This set-up induced territoriality in the males who could see each other and hence make them express full colour. Two Walimex pro Daylight 250S lamps were placed on either side of the cuvette and a first colour picture was taken of each fish with a Canon D60 camera equipped with a 50 mm lens (settings; P mode, ISO 200, auto focus). If a male failed to show territorial behaviour within an adjustment time of 1–2 days, it was moved back into its home tank and the process was repeated several weeks later. After good photos were obtained, each fish was sedated in MS222...
Figure 2. Phenotypic distributions in the two crosses. Distribution of absence (0; grey)/presence (1; red) scores for all traits in the (a) red-dorsum × blue cross and (b) red-chest × blue cross. A grey background indicates lack of variation in the F2 individuals in this trait (i.e. non-mappable traits), a red background indicates traits that are not differentiated between the two parental species but were variable in the F2 hybrids (transgressive traits). The sectors highlighted with colour on the inset cartoon fish are the traits that were mappable in the cross. Half red/half yellow indicates either colour could occur in this trait. (c,d) The distribution of the standardized phenotypic hybrid index, for the red-dorsum × blue cross (c) and for the red-chest × blue cross (d), which was calculated for every individual as the sum of presence scores in all traits differentiating the two parental species divided by the number of differentiating traits (see parts highlighted with colour in the inset fish figures). An index of 0 hence corresponds to the phenotype of the blue species, an index of 1 corresponds to the phenotype of the red species. Note that no red was scored on the nose on any F2 individual in the red-dorsum × blue cross. The comparison of the cartoon fish indicating mappable traits in (a,b) with the cartoon fish showing the parental types in (c,d) highlights which traits are transgressive in the F2 hybrids.
account for this, we repeated the single QTL analyses for the red-
sizes as compared to the red-dorsum × blue cross, and the two link-
could result in reduced power of detecting QTLs of similar effect
was calculated for each trait individually using the fitql function
the percentage of variation explained (PVE) of significant QTLs
ability coverage were calculated using the bayesint function, and
showing a significant effect of F1 family, mapping was additionally
0.05 as significant, and
were determined by permutations (n = 1000). For the red and
yellow traits, we used the binary model; for a multi-trait hybrid
mapping of male nuptial colour traits was performed in R/qtl
regression mapping algorithm with a LOD threshold of 1.0, a
were excluded. To build the linkage maps, we used the Kosambi
procedure was repeated five times in each cross.

3. Results
(a) Linkage maps
The final map for the red-dorsum × blue cross contains 232 mar-
kers in 22 linkage groups (corresponding to the number of
expected chromosomes in haplo-tilapiine cichlids [53,54]) with
an average marker distance of 5.3 cM and a total map length of
1117.8 cM. The final map for the red-chest × blue cross contains
1198 markers in 22 linkage groups with an average marker
distance of 1.2 cM and a total map length of 1360.8 cM.

(b) Phenotypic distributions
Seven traits differentiate the parental species in the red-
dorsum × blue cross (figure 2c): both parts of the dorsal fin,
both parts of the dorsum, head and nose are red versus blue,
and both parts of the flank (considered as one trait) are yellow
versus blue. However, nose was never scored as red in any F2.
Red was also scored in some F2s for both parts of the flank,
for gill cover, and for pelvic fin, although neither parental
species has red in these traits (figures 1 and 2c). Altogether
this resulted in 10 mappable traits for this cross (figure 2a).
The F2 phenotypes range from no red or yellow, respectively,
in any of the traits that differentiate the parental species (i.e.
like P. sp. ‘pundamilia-like’), to red or yellow, respectively,
in all of these traits with the exception of nose (i.e. like P. sp. ‘nyer-
erei-like’), with most individuals being intermediate in
expressing red in some but not all of these traits (figure 2c).
Ten traits differentiate the parental species in the red-chest ×
blue cross (figure 2d): the rear dorsal fin part, the frontal flank
part, the pelvic fin and all parts on the head (except for the
head part itself, which like the dorsum is greenish versus blue)
are red or yellow (flanks) versus blue. The rear dorsal fin part
was scored as red in all F2s and can hence not be mapped.
Some F2s were scored as red on the front part of the dorsal fin
as well as on both parts of the dorsum, on the head and on
the rear flank part, even though neither parental species is red
there (figures 1 and 2d). Altogether this resulted in 14 mappable
traits for this cross (figure 2b). The F2 phenotypes range from
no red or yellow, respectively, in any of these traits with the excep-
tion of the rear dorsal fin part (i.e. like P. pundamilia), to red or
yellow, respectively, in all these traits (i.e. like P. sp. ‘red-head’),
with most individuals being intermediate in expressing red in
some but not all of these traits. Yet, a large number of these F2
hybrids resemble P. sp. ‘red-head’ whereas fewer resemble
P. pundamilia, suggesting more directional dominance effects
in this cross than in the other cross.

(c) QTL mapping results
We found significant QTLs for red and yellow colour for seven
out of 10 mappable traits (figure 2a) in the sympatric red-

nyererei reference genome [45] with Bowtie2 v2.3.2 [46], allowing
one mismatch. This was followed by base quality recalibration
(see electronic supplementary material, appendix S2 for details)
and subsetting to uniquely aligned reads. GATK Unified Geno-
per [47] was used for genotyping (minimum base quality score set
to 20). The resulting vcf files were filtered with Bcftools
implemented in Samtools v.1.8 [48], Vcftools v.0.1.14 [49] and a
custom Python script, to obtain bi-allelic SNPs (see electronic
supplementary material, appendix S2 for details). For the red-
dorsum × blue cross, 368 SNPs were homozygous fixed in the
F0 and heterozygous in all four F1 parents, and were used in link-
age map construction. In the red-chest × blue cross, 2358 SNPs
were homozygous fixed in the F0 and heterozygous in two F1
parents (the other two F1s had low quality data that was removed
during filtering), and were used in linkage map construction.

(e) Linkage map construction
We used JoinMap 4.0 [50] to build linkage maps for both crosses.
We removed loci with extreme segregation distortion (p < 0.01),
loci with greater than 20% missing genotypes and identical loci
(i.e. SNPs within the same RAD locus) (greater than 0.950). Indi-
viduals were removed if they had greater than 30% missing data.
The linkage maps were generated from 216 F2 individuals (173
males, 43 females) in the red-dorsum × blue cross and 171 F2 indi-
viduals (115 males, 56 females) in the red-chest × blue cross. We
identified linkage groups based on an independent logarithm of
odds (LOD) threshold of 5. Loci with suspicious linkage (recombi-
nation frequency greater than 0.6) were removed. The strongest
cross-link (SCL) values in the maps are 4.7 (red-dorsum × blue
cross) and 4.6 (red-chest × blue cross), and unlinked markers
were excluded. To build the linkage maps, we used the Kosambi
regression mapping algorithm with a LOD threshold of 1.0, a
recombination threshold of 0.499, a goodness-of-fit threshold of
5.0 and no fixed order. We performed two rounds of mapping
with a ripple after addition of each marker to the map (see [50]).

(f) QTL mapping
QTL mapping of male nuptial colour traits was performed in R/qtl
[51,52]. We mapped the presence/absence of yellow colour on the
flanks and of red colour in 14 body and fin locations (traits; see
above and figure 2) in F2 males (n = 174 in the red-dorsum × blue
cross and n = 125 in the red-chest × blue cross) as binary traits.
Conditional genotype probabilities were calculated using the
calculgenoprobp function with a fixed stepsize of 1 (respectively 3
for scantwo; in cm), an assumed genotyping error rate of 0.05, and
the Kosambi map function. The scancme and scantwo functions
were used with the EM algorithm, and significance thresholds
were determined by permutations (n = 1000). For the red and
yellow traits, we used the binary model; for a multi-trait hybrid
index (see below), we used the normal model. We consider p <
0.05 as significant, and p < 0.1 as marginally significant. For traits
showing a significant effect of F1 family, mapping was additionally
performed with family as an additive covariate (allowing the aver-
age phenotype in the two families to be different) and as both an
additive and interactive covariate (additionally allowing the effect
of the QTL between the two families to be different). For significant
QTLs, approximate Bayesian credible intervals with a 0.95 prob-
ability coverage were calculated using the bayesint function, and
the percentage of variation explained (PVE) of significant QTLs
was calculated for each trait individually using the fitqtl function
(with the HK algorithm since the EM algorithm was not available
in this function for binary models).

The lower number of individuals in the red-chest × blue cross
could result in reduced power of detecting QTLs of similar effect
sizes as compared to the red-dorsum × blue cross, and the two link-
age maps differ substantially in number and density of markers.
To account for this, we repeated the single QTL analyses for the red-
dorsum × blue cross after randomly downsampling to 125 (of
174) individuals using the sample function in R to randomly pick
individuals and then subsetting the genotype–phenotype file to
these individuals. For the red-chest × blue cross, we repeated
the single QTL analyses after randomly downsampling markers
on the linkage map to match the number of markers on each linkage
group to those in our sparser red-dorsum × blue map, again
using the sample function in R to randomly pick markers within
each linkage group and then subsetting them to these markers.
The procedure was repeated five times in each cross.
For red in both sectors of the dorsal fin, the gill cover and the pelvic fin, we identified QTLs on Pun-LG8 with overlapping 95% confidence intervals, each with a PVE of 12.6–16.8%. A second QTL for red on dorsal fin sector 1 was found on Pun-LG10 with a PVE of 15.5%, and a second marginally significant QTL for red on the pelvic fin was found on Pun-LG12 with a PVE of 10.8%.

Two QTLs for red on both dorsum parts were identified, one on Pun-LG2 and one on Pun-LG6, both with a PVE of 12.4–17.4%. Pun-LG2 also contains a QTL for yellow flanks, within the 95% confidence mapping interval for red-dorsum, with a PVE of 29.5%. In this cross, only red in the pelvic fin (a transgressive trait in this cross) showed a significant effect of family \(F = 26.901, p < 0.001\). Repeating the mapping with family as covariate for pelvic fin recovered the QTL on Pun-LG8 and revealed an additional marginally significant QTL on Pun-LG22. We also found four QTLs for the phenotypic hybrid index (figure 3d), one each on LG2 (17.2% PVE), LG6 (8.5% PVE), LG8 (10.97% PVE), all of them within the 95% confidence interval for red-dorsum.

**Figure 3.** QTL mapping of the presence/absence of red (and yellow) male nuptial colour. LOD scores across the 22 Pundamilia chromosomes for all traits with presence scores for red and for yellow flanks (empty plots for non-mappable traits, figure 2) in (a) the red-dorsum × blue cross and (b) the red-chest × blue cross. The black dashed lines represent genome-wide significance thresholds of \(p < 0.1\) for each trait, the red dotted lines for \(p < 0.05\). (c) LOD scores across the chromosomes containing significant QTLs for red and yellow in the red-dorsum × blue cross (genome-wide significance thresholds of \(p < 0.05\) shown for each trait); (d) shows the chromosomes containing significant QTLs for the hybrid index in the red-dorsum × blue cross. See also electronic supplementary material, table S1 and figure S1.
confidence intervals of the QTLs found for the individual traits and one on LG18 (13.3% PVE).

Two-dimensional two QTL scans revealed several additional putative QTLs in this cross (electronic supplementary material, table S2). Repeating the single QTL mapping with 125 individuals randomly sampled from 174 five times recovered 82% of the expected total of 50 significant QTL results (i.e. 10 significant QTLs in the original dataset times five); 36 were recovered as significant and five as marginally significant (electronic supplementary material, table S3).

We found no significant QTLs for any of the 14 mappable traits (figure 2b) nor for the hybrid index in the non-sympatric red-chest × blue cross (electronic supplementary material, table S1; figure 3b). Several traits showed a significant effect of family in this cross: cheek (F = 16.478, p < 0.001), ventrum (F = 29.407, p < 0.001), upper lip (F = 29.079, p < 0.001), nose (F = 18.232, p = 0.001), throat (F = 46.281, p < 0.001) and yellow flanks (F = 5.6352, p = 0.025). Repeating the mapping with family as covariate for these traits; however, only detected one marginally significant QTL for red on the throat on Pun-LG7 with a PVE of 8.3%. A QTL for red on the head (a transgressive trait in this cross) reached the 0.1 significance threshold when repeating the mapping with a subsampled linkage map in two out of five such mapping rounds (electronic supplementary material, tables S1 and S4). Two-dimensional two QTL scans for this cross revealed two potentially interacting QTLs each for red on the cheek and throat (Pun-LG1 and Pun-LG4 for both traits) and for yellow on the flanks (Pun-LG3 and Pun-LG11) (electronic supplementary material, table S2).

4. Discussion

We investigated the genetic architecture of a trait complex of key importance to speciation in Lake Victoria cichlid fish, male nuptial colour motifs that feature importantly in behavioural reproductive isolation. In a cross between two sympatric species, representative in their mating trait motifs of many closely related sympatric species pairs, we found significant QTLs with moderate to large effects for red and yellow colour traits, with several traits mapped to the same genomic regions. These results are consistent with genetic architectures predicted to facilitate differentiation and persistence of differentiation in traits contributing to reproductive isolation in sympathy with ongoing gene flow [32–34,36–38]. By contrast, we did not find any significant QTLs in a cross between two species representative in their mating trait motifs of closely related species that are usually seen to occupy different islands but do not occur in sympathy. This is consistent with our hypothesis of a genetic architecture that makes phenotypic differentiation not robust to gene flow. We argue that these differences in genetic architecture of superficially similar trait differences could help explain why species with the red-dorsum nuptial colour motif are often sympatric with blue sister species, whereas those with the red-chest motif seem unable to retain differentiation from their blue relatives in sympathy.

The difference between the two crosses in the presence/absence of QTLs with moderate to large effects cannot simply be explained by a difference in power to detect QTLs: repeatedly and randomly downsampling the red-dorsum × blue cross F2 individuals to match the lower sample size in our red-chest × blue cross (a lower sample size decreases power to detect QTLs) did not significantly change the results, nor did downsampling the markers on the linkage map in the red-chest × blue cross to match the sparser red-dorsum × blue cross-linkage map (a sparser marker density lowers the significance threshold). Another statistical bias [55], where low sample sizes lead to overestimation of effect sizes or PVE, mainly due to the difficulties of statistically detecting loci with small effects, cannot be ruled out. However, this would be expected to affect both of our crosses and should thus not confound the comparison between the two. The different direction of our two crosses (red female × blue male in the red-dorsum × blue cross, blue female × red male in the red-chest × blue cross) should also not affect our results: the lab-strain populations from which the grandparents were taken have stable male colour. Also, none of the QTLs map to known sex determining chromosomes (Pun-LG10 in the red-chest × blue cross [45]), which is also consistent with earlier studies of experimental crosses of the same red-dorsum versus blue species pair [56–58]. Furthermore, in both crosses, both parental phenotypes (considering traits differentiating the two species, figure 2c,d) are recovered (with the exceptions that one trait (nose) was never scored as red in any F2s in the red-dorsum × blue cross, and one trait (dorsal fin 2) was scored as red in all F2s of the red-chest × blue cross). Additionally, most individuals are intermedrate in the number of traits in which red is expressed in both crosses, albeit with signs of more red dominance in the red-chest × blue cross.

A previous crossing experiment [56] estimated that the difference in the amount of red on the body (mostly flank and dorsum) of males between Pundamilia sp. ‘nyereke-like’ and Pundamilia sp. ‘pundamilia-like’ (our red-dorsum × blue cross) is likely controlled by at least 2–4 loci (with effects of dominance and epistasis). Furthermore, they estimated one gene with complete dominance for yellow flank and epistatic interaction with red on flank and dorsum. Our results conform to these estimates quite well: we found two significant QTLs each for red on the dorsum (LG2 and LG6) and for red on the dorsal fin (LG8 and LG10), each with a PVE of 12–17%. The interval of the QTL for red on the dorsum on LG2 also contains a major QTL for yellow on the flanks with a PVE of nearly 30%, and the significant QTLs for pelvic fin and gill cover overlap with the interval of the QTL on LG8 for red on the dorsal fin, suggesting either linkage of several loci or pleiotropic effects of a single locus. Two-dimensional 2-QTL scans indicate the presence of additional loci contributing to red/yellow colour. Although our modest sample sizes do not allow us to detect small effect QTLs, they are likely present, as our QTLs do not explain all of the variance in our mapped traits. However, the main contribution to variance in red and yellow male colour in this cross comes from these four QTL regions. A first screen of the genes closest to the QTL peaks has not yet revealed any obvious candidate genes. To follow-up on screening for candidate genes across the mapping intervals, each of which contains many dozens to hundreds of genes, will be a topic of future work.

The presence of moderate to large effect QTLs should make phenotypic differentiation and maintenance of differentiation in sympathy more likely than a more dispersed architecture of many loci with small effects under the opposing effects of disruptive selection and gene flow e.g. [32–34,38]. Furthermore, the theoretical models of sympatric
speciation by sexual selection suggest such a process is more likely when reproductive isolation is based on traits underlain by fewer loci [39–41]. Additionally, we find that several traits (figure 3c) map to the same chromosomal region. Although our current dataset does not allow us to determine whether this is due to a single pleiotropic locus or due to several tightly linked loci, both pleiotropy and physical linkage favour divergence and persistence of phenotypic differentiation despite gene flow [34,36,37]. Linkage (or pleiotropy) of divergent adaptive traits has also been observed in Midas cichlids, which are undergoing rapid sympatric divergence [59]. Our findings are also similar to other systems such as Heliconius [60], Mimulus [61] or Drosophila [62], in which traits involved in reproductive isolation in the presence of gene flow are underpinned by large effect or pleiotropic loci. However, none of these other studies made a direct comparison of the genetic architecture of corresponding key traits for reproductive isolation in species pairs that persist (and probably evolved) in sympathy and others that do not persist in sympathy, as we have done here.

In our cross between the species that do not persist in sympathy, i.e. Pandanamilia sp. ‘red-head’ and Pandanamilia pandanamilia (red-chest × blue), we cannot directly infer the type of genetic architecture underpinning red, or yellow due to the absence of any significant QTLs. Most likely, the effects of potential QTLs are too small to be detected with our sample size, suggesting the presence of a larger number of small effect loci. In red-chest versus blue species without direct geographical contact and with little gene flow that is restricted to rare (but documented, figure 1) long distance dispersal events, trait differentiation due to more and smaller effect mutations is more likely to evolve and persist because recombination will not erode associations between them.

What we cannot yet resolve is whether the genetic architecture for male colour in our red-dorsum versus blue pair has evolved in the face of gene flow, i.e. through selection for male colour in our red-dorsum versus blue pair recombination will not erode associations between them. An effect mutations is more likely to evolve and persist because reproductive isolation in species pairs that persist (and probably evolved) in sympathy and others that do not persist in sympathy, as we have done here.

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What we cannot yet resolve is whether the genetic architecture for male colour in our red-dorsum versus blue pair has evolved in the face of gene flow, i.e. through selection for the clustering of small effect loci, for instance through genomic rearrangements [63], or was already present, allowing the two species to speciate (and now persist) in sympathy.

5. Conclusion

The presence of large effect loci, and of physical linkage or pleiotropy, underlying traits involved in behavioural reproductive isolation, such as male nuptial coloration, may enable sister species pairs to differentiate and persist in sympathy despite some gene flow. One hallmark of the East African cichlid radiations is the rapid evolution of strong (behavioural) reproductive isolation that is robust to full sympatry in many closely related species [64]. If the genetic architecture of male nuptial coloration in sympatric Pandanamilia species we report here is representative for other Lake Victoria cichlid species that live sympatrically, this may help explain how speciation in this system could have led to the rapid emergence of communities with many closely related species that persist in sympathy, and why some phenotypic motifs regularly distinguish sympatric species while others are confined to allopatric species.

References


17. Hamilton SE. 2016 Creation of a bathymetric map of Lake Victoria, Africa. See http://dx.doi.org/10.7910/DVN/25WZA.


