

Research



Cite this article: van Rijssel JC, Moser FN, Frei D, Seehausen O. 2018 Prevalence of disruptive selection predicts extent of species differentiation in Lake Victoria cichlids.

Proc. R. Soc. B **285**: 20172630.

<http://dx.doi.org/10.1098/rspb.2017.2630>

Received: 24 November 2017

Accepted: 2 January 2018

Subject Category:

Evolution

Subject Areas:

evolution, ecology

Keywords:

assortative mating, character displacement, disruptive selection, evolutionary response, fitness surface, speciation with gene flow

Authors for correspondence:

Jacco C. van Rijssel

e-mail: jacco.vanrijssel@wur.nl

Ole Seehausen

e-mail: ole.seehausen@eawag.ch

[†]Present address: Wageningen Marine

Research, Wageningen University & Research, IJmuiden, The Netherlands.

Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.figshare.c.3972825>.

figshare.c.3972825.

Prevalence of disruptive selection predicts extent of species differentiation in Lake Victoria cichlids

Jacco C. van Rijssel^{1,2,†}, Florian N. Moser^{1,2}, David Frei^{1,2} and Ole Seehausen^{1,2}

¹Department of Fish Ecology and Evolution, EAWAG Center of Ecology, Evolution and Biogeochemistry, Kastanienbaum, Switzerland

²Institute of Ecology and Evolution, University of Bern, Bern, Switzerland

JcVr, 0000-0001-8038-9674; FNM, 0000-0003-4542-6956

Theory suggests that speciation with gene flow is most likely when both sexual and ecological selection are divergent or disruptive. Divergent sexual and natural selection on the visual system have been demonstrated before in sympatric, morphologically similar sister species of Lake Victoria cichlids, but this does not explain the subtle morphological differences between them. To investigate the significance of natural selection on morphology during speciation, we here ask whether the prevalence of disruptive ecological selection differs between sympatric sister species that are at different stages of speciation. Some of our species pairs do (*Pundamilia*) and others do not (*Neochromis*) differ distinctively in sexually selected male nuptial coloration. We find that (i) evidence for disruptive selection, and for evolutionary response to it, is prevalent in traits that are differentiated between sister species; (ii) prevalence of both predicts the extent of genetic differentiation; and (iii) genetic differentiation is weaker in species pairs with conserved male nuptial coloration. Our results speak to the existence of two different mechanisms of speciation with gene flow: speciation mainly by sexual selection tightly followed by ecological character displacement in some cases and speciation mainly by divergent ecological selection in others.

1. Introduction

Ecological speciation is the process by which adaptation to different ecological niches results in reproductively isolated sister species. This can, in principle, occur in a range of geographical settings [1–3]. Yet, despite much interest, and despite an abundance of evidence for allopatric and parapatric incipient species, compelling examples of ecological speciation that is robust to sympatry, are not very many [4–6] and are concentrated in few taxonomic groups [1,5]. Because return to sympatry of sister species during or soon after speciation is of fundamental importance for the rate of species accumulation [7], understanding causes of variation in evolution and persistence of early stage species differentiation in sympatry is of critical importance.

There are many possible explanations for the relative rarity of ecological speciation that is robust to sympatry, henceforth referred to as sympatry-robust speciation. We define sympatry-robust speciation as when early stages of species differentiation (i.e. incipient speciation) persist in narrow sense sympatry (i.e. within the same habitat). This is distinct from the much more often studied, and probably more widespread parapatric ecotype formation where divergence is contingent upon occupation of spatially discrete non-overlapping habitats, such as between lake and stream ecotypes of fish. However, in contrast to sympatric speciation, our definition of sympatry-robust speciation does not require that the divergence process has begun in sympatry. So, why is it relatively rare? First, whereas divergent selection between allopatric or parapatric populations occupying different habitats may be common, disruptive selection in sympatry may be rare. When individuals in a population can exploit more than one type of resource without experiencing strong performance trade-offs, generalist strategies may be

favoured [8]. When distinct resources require very different adaptations and when a population is sufficiently narrow in its resource use such that individuals cannot tap into alternative resource types even though such exist, the population will not experience disruptive selection either. We refer to both cases as 'lack of disruptive selection' scenarios.

Second, even when individuals within populations do experience performance trade-offs, ecological speciation may still not occur or not be sympatry-robust because populations may escape the fitness trough by mechanisms of adaptation that do not require reproductive isolation. These include, first, the evolution of ecological dimorphism between the sexes [9], favoured when there are no developmental or ecological constraints on phenotypic divergence of the sexes. Second, evolution of a major effect gene with dominance (Mendelian switch) where every genotype resides under one of the alternative fitness optima (suggestive examples are *Pyrenestes* finches [10], scale eating cichlids [11] and the mimicry polymorphic butterfly *Heliconius numata* [12]). Third, the evolution of adaptive phenotypic plasticity [13], favoured when reliable environmental cues meet a low cost of plasticity [8]. In all of these cases, phenotypic adaptation is possible despite free gene flow. We refer to these scenarios collectively as 'adaptation without speciation' scenarios.

Finally, when a population does experience disruptive ecological selection and when none of sexual dimorphism, Mendelian switches nor adaptive plasticity can evolve to dissipate this selection, adaptation cannot occur unless assortative mating evolves because random mating and recombination will destroy linkage disequilibria between physically unlinked genes favoured by selection because together they generate alternatively adapted phenotypes. We refer to this as the 'failed adaptation scenario'.

Any and all of these scenarios may contribute to the rarity of sympatry-robust speciation. Constraints to the evolution and maintenance of linkage disequilibria against gene flow is classically thought to be the main reason for the rarity of sympatric speciation in the strict sense [14] and may also explain the rarity of species persistence upon early return to sympatry when reproductive isolation is still incomplete. Theory suggests that such speciation is possible though and is most likely when both ecological and sexual selection are divergent and synergistically interact, which may facilitate the evolution of assortative mating [2,15–18]. In this paper, we begin to test predictions of some of these hypothetical explanations for variation in the extent of sympatry-robust speciation among Lake Victoria cichlid fish.

African cichlid fish are a textbook example of adaptive radiation, and speciation without geographical isolation occurred in approximately half of the lakes where these fish reside [19]. Adaptive radiation is especially prevalent in the haplochromine group, where even very young sister species can often coexist in sympatry despite gene flow [20,21]. However, haplochromine lineages vary in their extent of diversification, and replicate cases of speciation within the same lineage vary in the extent of species differentiation evolved or maintained in sympatry. In Lake Victoria, replicate cases of sympatric phenotypic polymorphisms, incipient and sister species persist to date at various stages of differentiation, representing different stages of sympatry-robust speciation [20,22]. Here we investigate whether differences in present day ecological selection regimes help explain some of this within-lineage variation.

We studied selection in seven assemblages of phenotypically variable populations of rock-dwelling cichlids that differ in the extent of phenotypic, ecological and genetic differentiation: two genetically panmictic, but phenotypically variable populations and five pairs of sympatric incipient and sibling species that range from weak to strong, albeit incomplete reproductive isolation [20,22,23]. In the following, we refer to them collectively as 'form pairs'. We ask whether variation in the extent of differentiation among these form pairs may be explained by variation in the form and prevalence of ecological selection.

One way of estimating ecological selection on variable phenotypes is by inferring phenotypic fitness surfaces from the relationship between trait values and a fitness proxy [24]. In fish, individual variation in growth rate is an often-used surrogate measure of fitness variation [25–28]. Individual growth rate variation within populations of fish tends to be highly positively correlated with food intake, and although growth rate is not strictly a fitness component, faster growth has several positive fitness consequences [29] such as higher survival due to predation avoidance by gape-limited predators [30], earlier breeding [31] and higher fecundity [32]. For Lake Victoria cichlids, higher growth rate can additionally provide advantages in foraging, territoriality and mate choice. Larger males may have an advantage in male territorial competition [33] that can result in higher quality territories [34,35], the latter being crucial for feeding and male reproductive success [35].

In this study, we (i) ask whether present day disruptive selection acts on those traits that differ between sympatric forms, and hence if these differences could have evolved under disruptive selection, and (ii) test the hypothesis that differences between replicate pairs in the prevalence of disruptive selection explains variation in the extent of phenotypic and genetic differentiation between sympatric forms.

Male nuptial coloration is strongly differentiated between forms in three of our replicate form pairs, where it is associated with divergent female preferences, assortative mating and genome-wide differentiation. Conspicuous differences in male coloration are lacking and behavioural assortative mating, measured in the laboratory, is absent in the other four. Before we outline the methods, we introduce the study system and the sympatric form pairs.

(a) Study system

Haplochromine cichlid fish in Lake Victoria have diversified into more than 500 endemic species [36] mostly within the past 15 000 years [37]. Lake Victoria haplochromines have strongly sexually dimorphic coloration with colorful males and cryptic females [38]. Male coloration in these fish is under sexual selection by female mate choice [35] and male–male competition [33,39], and is important in the evolution of behavioural reproductive isolation and speciation in many cases [23,40,41]. With few exceptions, closely related species have similar ecologies (i.e. they belong to the same trophic and habitat guilds), but they are rarely ecologically identical, which is reflected in subtle differences in morphology [42].

(b) Species complexes

We studied four sympatric form pairs in the genus *Pundamilia* at the islands of Luanso, Kissenda, Python and Makobe, and three sympatric form pairs in the genus *Neochromis* at the islands of Bihiru, Igombe and Makobe, all in southeastern Lake Victoria

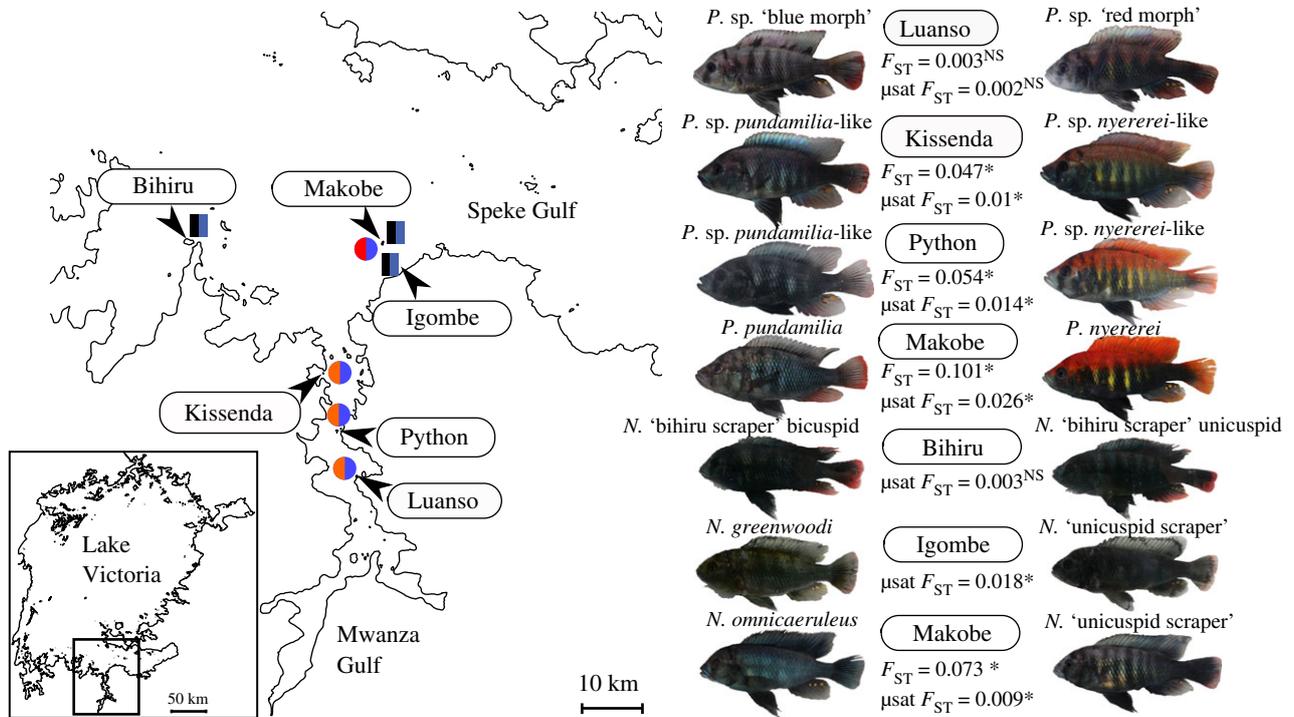


Figure 1. Map of Lake Victoria and the Mwanza Gulf indicating sampling sites, and the seven form pairs at different stages of speciation. Half blue and red circles indicate islands where the *Pundamilia* pairs occur, the half blue and half black rectangles indicates the occurrence of the *Neochromis* pairs. Genomic divergence is based on RAD tag F_{ST} -values from Meier *et al.* [20] and Brawand *et al.* [43], and on microsatellites F_{ST} -values from Seehausen *et al.* [23] and Magalhaes *et al.* [22]. Asterisks indicates a significant F_{ST} -value.

(figure 1). Morphologically similar sympatric species of *Pundamilia* (*P. pundamilia* and *P. nyererei* at Makobe, *P. sp. pundamilia*-like and *P. sp. nyererei*-like at Python and Kissenda [20]) differ strikingly in male nuptial coloration and species-specific female mating preferences are based on these differences [44]. By contrast, the colour differentiation in the *Neochromis* form pairs is less distinct but they are more distinct ecologically and in their associated dentition and tooth morphology [22]. Whereas both sympatric *Pundamilia* species feed mostly on insects and zooplankton (albeit at different proportions) and have nearly identical tooth morphology, one taxon in each of the *Neochromis* pairs scrapes algae from rocks and possesses many rows of densely spaced bicuspid teeth, while the sister taxon has more widely spaced unicuspid teeth and feeds mainly on cyanobacteria in less sunlit habitats [22]. The *Neochromis* species do not differ conspicuously in male coloration and do not mate assortatively in laboratory mate choice trials (O. Selz & O. Seehausen 2016, unpublished data), yet their genetic differentiation at two of our islands [22] is comparable with that between the *Pundamilia* species at Kissenda, Python and Makobe Islands [20,23] (figure 1).

We expect that, in the absence of evidence for behavioural assortative mating and no obvious divergent sexual selection, disruptive ecological selection should be more prevalent in *Neochromis* than in *Pundamilia* taxon pairs to permit a similar extent of genomic differentiation. In both genera, we sampled form pairs at different stages of differentiation [20,22,23] (figure 1; electronic supplementary material, appendix S1).

2. Material and methods

(a) Study sites

Fish were collected from six different islands in the Mwanza and Speke Gulfs of the southern Lake Victoria: Luanso, Kissenda,

Python, Makobe, Igombe and Bihiru (figure 1). For details on the habitat at these islands, see [22,23,44] (electronic supplementary material, appendix S2). All fish were collected during the autumn of 2014, except for *Neochromis* at Igombe and Bihiru islands that were collected in the autumn of 2005.

For the calculation of fitness surfaces, we measured only males: 51 *Pundamilia* from Luanso, 228 from Kissenda, 170 from Python, 144 from Makobe, 62 *Neochromis* from Bihiru, 38 from Igombe and 195 from Makobe (electronic supplementary material, table S1). Where sample sizes of the sympatric forms were uneven (due to differences in abundance and catchability), we subsampled the individuals of the more numerous species (*P. nyererei* at Makobe Island and *P. sp. nyererei*-like at Python and Kissenda Islands) down to 10 or 25 fish per 2 m depth interval, respectively, for Makobe or Python and Kissenda. For the other species, we used all fish available. We measured only males because females are difficult to identify with confidence between incipient species, and impossible to identify to colour morphs at Luanso Island.

(b) Morphological measurements

We measured 14 established, ecologically relevant morphometric distances using a digital caliper [45]: standard length (SL), body depth (BD), head width (HW), head length (HL), snout width (SnW) and length (SnL), lower jaw width (LJW) and length (LJL), eye depth (EyD) and length (EyL), cheek depth (ChD), pre-orbital depth (POD) and width (POW) and inter-orbital width (IOW). We log-transformed and size-corrected all of them. We also counted the number of tooth rows in the upper oral jaw (Rows) and the number of teeth in the outermost tooth row of the upper oral jaw (Teethnr). We calculated the percentage of unicuspid (% Uni), bicuspid (% Bi) and tricuspid (% Tri) teeth among the teeth in the outermost row of the upper oral jaw (electronic supplementary material, appendix S3).

(c) Stable isotopes

As a measure of trophic ecology and to ask if selection would act directly on trophic ecology, we analysed stable isotope ratios of

$\delta^{13}\text{C}$, indicating relative importance of benthic versus limnetic carbon sources [46], and $\delta^{15}\text{N}$, indicating relative trophic position [47]. The $\delta^{15}\text{N}$ signature of consumers are typically enriched with 3–4‰ with each trophic level while the $\delta^{13}\text{C}$ signatures are not affected by trophic level or only slightly enriched ($\delta^{13}\text{C} < 0.1$ ‰ [48]) (electronic supplementary material, appendix S4).

(d) Fitness proxy

We estimated the age of individuals by counting the number of scale circuli of one scale for each specimen (electronic supplementary material, appendix S5). Scale circuli are bony rims which are added to the interior margin of each scale as the fish grows [49]. In young juveniles, these circuli are created on a daily basis but the deposition rate decreases as the fish becomes older [50,51]. The number of scale circuli is correlated with age [51]. We conducted a growth experiment in the laboratory using first generation laboratory-bred *P. pundamilia* from Makobe to verify our age estimate; number of scale circuli was significantly positively correlated with age ($r = 0.26$, $p = 0.04$; electronic supplementary material, appendix S5 and figure S1).

To estimate individual growth rates, we performed pooled within-group regressions (species and islands) of the log-transformed SL against the number of circuli (age), the residuals of these regressions were used as estimates of growth rate. Our growth rate estimate showed a significant positive correlation with actual growth rate measured in our growth experiment ($r = 0.59$, $p < 0.001$; electronic supplementary material, appendix S5 and figure S1). To estimate growth rates of individuals in the phenotypically variable but genetically panmictic populations at Luanso and Bihiru Islands, and for individuals of intermediate phenotype from other islands, we performed within island regressions, including all fish from one form pair of this island (electronic supplementary material, figure S2).

(e) Selection analyses

To estimate the form and strength of selection on morphological and ecological traits (water depth utilization and the two stable isotopes), standardized growth rate residuals and trait residuals were used to obtain selection gradients using a range of different methods. First, we estimated directional, correlational and quadratic selection for ecological traits (water depth, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and for morphological traits using the ordinary least-squares regression approach of Lande & Arnold [24] and doubled the quadratic regression coefficients [52] (see electronic supplementary material, appendix S6 for details on the selection analyses). Because this method works best for large sample sizes (greater than 500) [53] that can not be acquired for local cichlid populations in the wild, we additionally estimated the statistical relationships between individual traits and fitness by applying the `lm` function in R where a single trait was used as predictor variable and growth rate as the response variable. The regression coefficients of these models have to be interpreted with caution because they may represent either selection gradients or apparent selection gradients due to genetic, developmental or functional correlation between traits [24,54]. In these models, disruptive selection is generally indicated by positive, and stabilizing selection by negative quadratic coefficients of growth rate on phenotype.

Because fitness surfaces may be more complex [54], polynomial regressions up to the fourth degree were used to test the relationship between growth rate and each morphological and ecological trait and were estimated using the `lm` function and plotted using the `predict` function in R [55]. The relative goodness of fit of each model was tested with the AICc statistic. The ΔAICc was calculated by subtracting the AICc of the linear model from that of the significant quadratic, 3rd or 4th order model. We considered a model fitting the data significantly better when ΔAICc was less than -2.5 or greater than 2.5 , where negative values indicate a

better fit for the linear model (uniform directional selection) and positive values indicate a better fit for the quadratic, 3rd or 4th order model (stabilizing or disruptive selection). For data with multiple or without significant polynomial models, we compared the linear model with the polynomial model that had the lowest AICc. More often than not, phenotypic selection works on multiple traits that may or may not be phenotypically correlated. To control for effects of phenotypic correlation, we calculated trait correlations within each island population of every species and reduced the set of traits to those that were uncorrelated (electronic supplementary material, figures S3, S4). For example, when trait A correlates with trait B, and B correlates with trait C, but A and C are not correlated, we only retained trait B. Among correlated traits, we retained only the one with the larger regression coefficient. To visualize the number of individuals on inferred fitness peaks, Kernel density estimates were plotted with the density function in R. Because the sample sizes for the different taxon pairs differed between locations, with rather low ($n = 38$) sample sizes for the smallest population, we performed bootstrapping analyses, to check the loss of power to detect disruptive selection with decreasing samples size (electronic supplementary material, appendix S6).

Selection might often act on multiple traits simultaneously [56]. Because we were interested in divergent or disruptive selection between the sympatric forms, we performed a canonical analysis and subsequently an eigenanalysis to find the multivariate axes of highest quadratic curvature relative to growth rate. We also fitted project pursuit regressions to each island assemblage (electronic supplementary material, appendix S6) [57,58]. Contour plots of fitness as a function of the trait combinations, M matrices and the projection pursuit regression analyses were visualized as fitness surfaces using the `Tps` function in the R package `fields`.

Finally, evolutionary response to selection was quantified for traits or trait combinations for which we inferred disruptive selection. Evolutionary response to disruptive selection, consistent with a role in species divergence, was inferred for a trait or a combination of traits when (i) at least two fitness peaks, separated by a valley lower by at least 0.2 than the lower one of the peaks were occupied by a minimum of three individuals per peak and when (ii) the majority of the individuals occupying one fitness peak (greater than or equal to 70%) were from one species/morph while the majority (greater than or equal to 70%) of individuals occupying the other peak were from the other species/morph.

3. Results

(a) Divergent morphological and ecological traits

Among the *Pundamilia*, the morph pair from Luanso Island showed the least developed trait differentiation with none of the morphological traits being significantly divergent. The magnitude of trait divergence was intermediate in the incipient species pairs of Kissenda and Python Islands. At Python, nine morphological traits were significantly differentiated, three trait means being more divergent here than in any of our other *Pundamilia* pairs. At Kissenda, 12 morphological traits were significantly differentiated, also here, three trait means being more divergent here than in any of our other *Pundamilia* pairs. The species pair from Makobe Island showed the greatest magnitude in trait differentiation, with 10 traits significantly differentiated of which eight show the largest difference between mean trait values (electronic supplementary material, appendix S7, table S2).

Among the *Neochromis*, the morph pair at Bihiru differed in four traits but none of these differences were larger than in

the other *Neochromis* form pairs. The incipient species pair at Igombe showed the greatest trait differentiation in six morphological traits of a total of 10 differentiated traits. The species pair of Makobe has the highest number of differentiated traits (15) and again shows the greatest magnitude in trait differentiation with eight out of 17 morphological traits, as well as water depth occupation showing the largest mean trait difference among all our *Neochromis* (electronic supplementary material, appendix S7, table S2).

(b) Selection on individual traits

Using the Lande & Arnold approach, which takes the correlation of traits into account, we found evidence for significant disruptive selection on two morphological traits (HL and POW) in the *Pundamilia* pair at Python Island, and for one trait (LJW) in *Pundamilia* at Luanso Island (electronic supplementary material, table S6). All of these traits are significantly differentiated in at least one of the sympatric *Pundamilia* form pairs, and all are diverging in the same direction between the blue and the red forms in all cases (electronic supplementary material, table S1). For the *Neochromis* pairs, we found evidence for significant disruptive selection on one morphological trait (HL) at Makobe Island and also for one (BD) at Bihiru Island (electronic supplementary material, table S9). Both of these traits are significantly differentiated between several of our *Neochromis* pairs, but not always in the same direction (electronic supplementary material, table S1).

In our univariate trait selection analyses, the *Pundamilia* form pairs from Luanso, Kissenda and Python, and the *Neochromis* form pair from Bihiru all show evidence of disruptive selection on just a few morphological and/or ecological traits. Only in the *Pundamilia* pair from Python Island do these traits correspond to traits that have significantly differentiated trait means between the two species, and only in one of these traits there is evidence consistent with an evolutionary response to this selection (figures 2 and 3; electronic supplementary material, appendix S8, table S3). The *Pundamilia* species pair from Makobe and the *Neochromis* species pairs from Makobe and Igombe, on the other hand, show considerable evidence of disruptive selection on a larger number of traits (electronic supplementary material, table S3). In these pairs, we also see greater prevalence of disruptive selection on traits that are differentiated between sympatric sister species, and stronger evidence for divergent evolutionary response (figures 2 and 3; electronic supplementary material, appendix S8, table S3). Given our measures of genetic differentiation in all seven form pairs (figure 1), it appears from our univariate selection analysis that a larger number of traits show signatures of disruptive or apparent disruptive selection in taxon pairs with stronger genetic differentiation than in those with no or only little genetic differentiation.

(c) Correlational selection

The number of paired trait combinations with evidence for disruptive selection was none out of 28 for *Pundamilia* Luanso, none for Kissenda, one for Python and none for Makobe (out of 91 combinations in each of these pairs; electronic supplementary material, appendix S9). No evidence for evolutionary response was apparent in the one trait combination that showed evidence of disruptive selection in the Python taxon pair (HW-POD) (electronic supplementary material, tables S7–S9, figure S22). For *Neochromis* Bihiru, we

found evidence of disruptive selection in three out of 21 trait combinations and in one of these combinations (BD-POD) evidence for a divergent evolutionary response was apparent. No evidence of disruptive selection was found for any of 28 trait combinations in *Neochromis* Igombe. For *Neochromis* Makobe, evidence of disruptive selection was evident in 10 out of 91 trait combinations, five of which also showed patterns consistent with divergent evolutionary responses (HL-EyD/IOW/SnW, depth- $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$; electronic supplementary material, appendix S9, tables S8–S10, figure S22). In short, the number of paired trait combinations showing evidence of disruptive selection and evolutionary response appears higher in *Neochromis* than in *Pundamilia* (electronic supplementary material, table S3).

(d) Multivariate selection

In each of our form pairs growth rate showed significant quadratic relationships with almost all the morphological eigenvectors the canonical analyses (electronic supplementary material, tables S11, S12). The three eigenvectors with the largest eigenvalues showed a mix of evidence for weak disruptive and stabilizing selection in all cases. However, some of the traits that loaded heavily on the major vector under disruptive selection were the same in several form pairs (e.g. LJL in *Pundamilia*) (λ ; electronic supplementary material, figure S23, table S11, S12). By contrast, no significant linear relationships were found between growth rate and eigenvectors (electronic supplementary material, tables S11, S12).

In both *Pundamilia* and *Neochromis*, evidence for disruptive selection and evolutionary response to it were more pronounced in taxon pairs that are more strongly genetically differentiated, and more in *Neochromis* than in *Pundamilia* (electronic supplementary material, appendices S10, S11, tables S3, S11–S13, figures S23, S24). This is consistent with the results of the selection analysis on individual traits (electronic supplementary material, table S3).

4. Discussion

Related questions in speciation research are why sympatric and sympatry-robust speciation is generally rare in animals, and why it is clearly more common in some taxa than in most others. Here, we studied sympatric replicate pairs of early stage speciation in a lineage of Lake Victoria haplochromine cichlid fish in which sympatry-robust speciation occurs much more often than in most other animals. By comparing replicate cases at different stages of species differentiation, we hope to learn about constraints to sympatry-robust speciation.

We found evidence for disruptive selection on morphological and ecological traits in most of our speciation replicates. Disruptive selection seemed to be mostly acting on traits that are differentiated between sympatric species, with indications of divergent evolutionary responses to this selection in several cases. Overall, disruptive ecological selection or apparent selection (see below) seemed to be more prevalent in pairs that are more strongly reproductively isolated (stronger genetic differentiation), and evolutionary response to such selection was only seen in genetically significantly differentiated incipient or sibling species. Evidence for disruptive selection and evidence consistent with a divergent

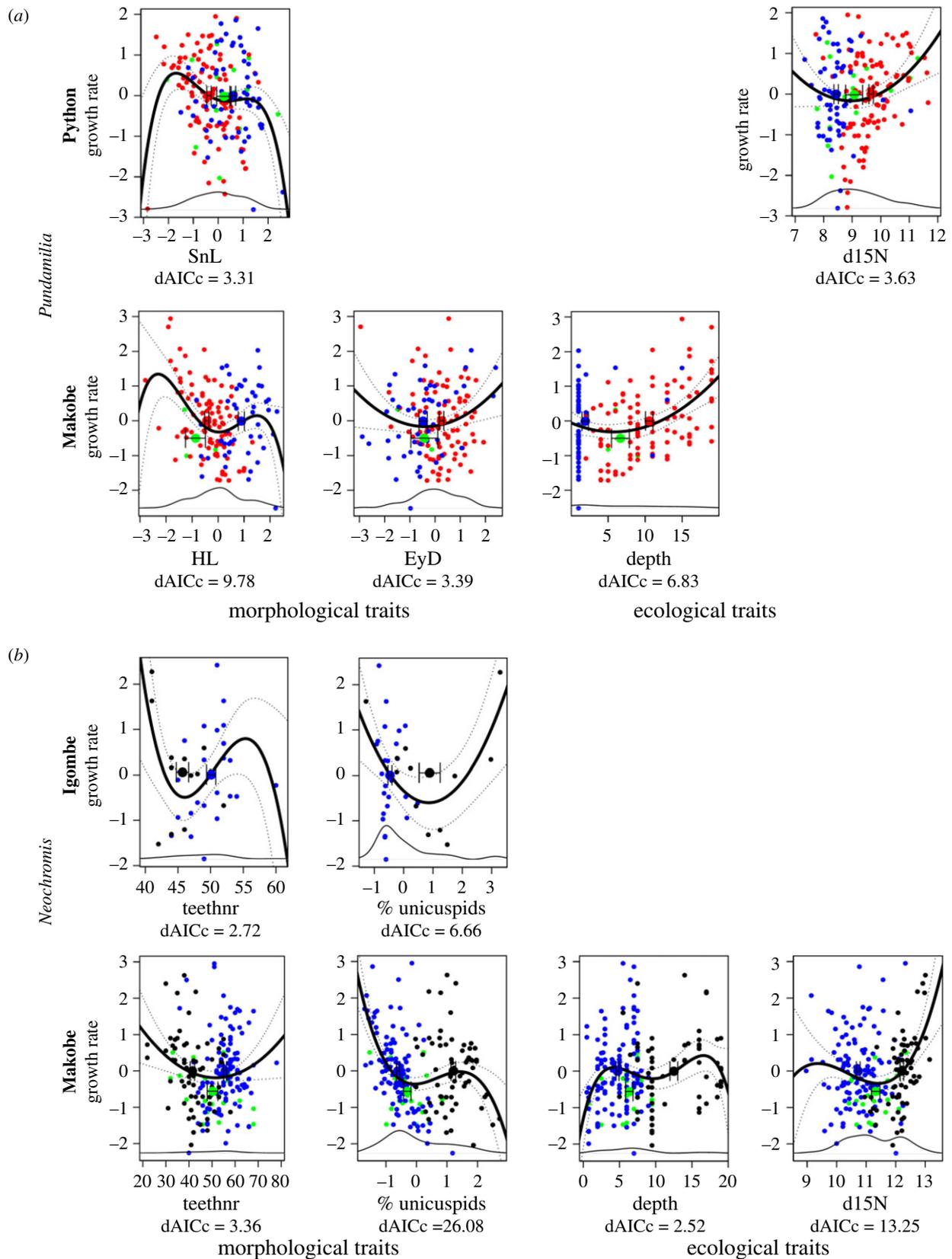


Figure 2. Fitness surfaces for the (a) *Pundamilia* and (b) *Neochromis* form pairs. Only surfaces shown that imply disruptive selection on univariate morphological or ecological traits that differ between the two species and that have a significantly better fit of the quadratic, 3rd or 4th order model compared to the linear model (dAICc of greater than 2.5) using complete sample sizes, all other data are shown in figures S14–S20 of the electronic supplementary material. Density of individuals at each trait value is indicated with thin black lines. For *Pundamilia*: red dots, *P. nyererei* and *P. sp. nyererei*-like; blue dots, *P. pundamilia* and *P. sp. pundamilia*-like. For *Neochromis*: black dots, *N. 'unicuspid scraper'*; blue dots, *N. omnicaeruleus/N. greenwoodi*; green dots, intermediate phenotypes. Large dots indicate average trait values and growth rate averages per species, error bars indicate trait standard deviations.

evolutionary response to it were both more prevalent in pairs within the genus *Neochromis*, which lack conspicuous differences in male nuptial coloration, than in pairs within

the genus *Pundamilia*, which are strongly differentiated in male breeding coloration and species-specific female mating preferences for these [59].

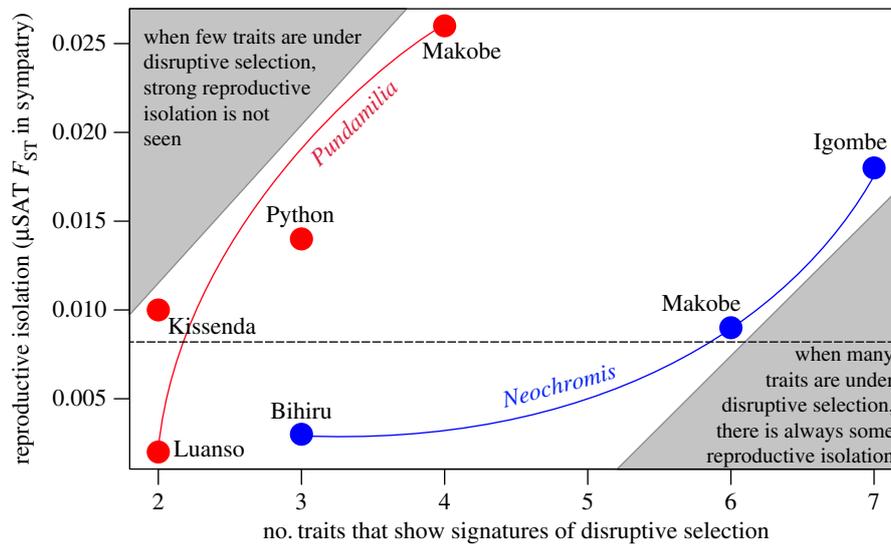


Figure 3. Plot of genetic divergence versus the number of univariate morphological and ecological traits showing evidence of disruptive selection. Genetic divergence is based on microsatellites [22,23]. The plot illustrates two different putative speciation mechanisms; for *Pundamilia* (red), the number of traits under disruptive selection might increase only when reproductive isolation, possibly induced by sexual selection, reaches a certain threshold, for *Neochromis* (blue), a relatively large number of traits under ecological disruptive selection might be necessary to establish and maintain reproductive isolation. The dashed line separates the form pairs with a significant F_{ST} -value from the ones without a significant F_{ST} -value.

Because we did not have the statistical power to demonstrate that our univariate selection gradients result from direct selection on each individual trait as opposed to at least some resulting from effects of multivariate correlational selection, we caution readers that some of our selection gradients may in fact represent apparent selection. Our species pair replicates are all very closely related to each other (none of these have diverged for more than a few thousand generations and occasional gene flow between all of them is possible and likely [20]) and are expected to share very similar trait variance–covariance matrices. We therefore think that our comparisons between replicate species pairs of the number of traits showing signatures of disruptive selection are speaking to overall variation in the prevalence of selection, both if differences reflect variation in the number of traits under selection independently, or variation in the number of traits drawn into effects of correlational selection.

With this in mind, our results are consistent with the hypothesis that variation in the extent of realized disruptive ecological selection helps explain variation in whether sympatry-robust speciation occurs or not. Our data further indicate that the presence of divergent sexual selection and behavioural assortative mating between forms facilitates speciation, whereas in its absence, stronger disruptive ecological selection may be required to achieve similar levels of reproductive isolation. In the following, we will discuss both of these tentative conclusions.

(a) Why does sympatry-robust speciation not happen in some taxon pairs despite phenotypic variation?

Independent of the amount of phenotypic variation ($p = 0.41$; electronic supplementary material, figure S21) across the replicate form pairs, evidence for disruptive natural selection seemed much more prevalent in some pairs than in others. The number of traits with evidence of disruptive selection tended to increase with increasing genetic differentiation ($r = 0.94$, $p = 0.06$ for *Pundamilia*; $r = 0.92$, $p = 0.25$ for *Neochromis*). Evidence for disruptive selection was least

prevalent in the genetically panmictic populations. Evidence for evolutionary response to the detected selection patterns followed a similar trend: stronger responses in genetically more strongly differentiated pairs. We therefore suggest that the prevalence of disruptive ecological selection (lack of disruptive selection scenario), as well as the prevalence of an evolutionary response to selection (failed adaptation scenario) both help to predict the stage of speciation in these sympatric pairs.

Although we see no evidence for balanced phenotypic polymorphisms in these specific cases, we cannot fully exclude the possibility of the ‘adaptation without speciation scenario’ more broadly speaking. For instance, ecological dimorphism between the sexes might allow for adaptation without reproductive isolation. Morphological dimorphism between sexes is known in several of our taxon pairs [42]. Measuring how selection acts differently between the sexes would be very interesting. However, for the evolution of ecological dimorphism between the sexes to explain the lack of speciation, dimorphism would have to be stronger at the islands where sympatry-robust speciation has not happened. There is no evidence for this (if anything, trends may point the opposite direction). Adaptation without reproductive isolation can also be achieved through adaptive phenotypic plasticity. Although adaptive plasticity is a known phenomenon in cichlids [60], most traits that showed evidence of disruptive selection in our data have a large heritable component, such as HL, L JL (electronic supplementary material, appendix S12) in *Pundamilia* [61] and tooth shape in *Neochromis* [22], suggesting that at least for these traits, plasticity is unlikely to explain our data. In addition, Meier *et al.* [62] found that, out of 34 candidate genes of parallel divergent selection within the *Pundamilia* pairs of Makobe and Python, five are involved in limb, bone and cartilage development and thus may be involved in the morphological differences between *Pundamilia* species. Moreover, if adaptive plasticity was to explain the absence of speciation in some of our form pairs, we would expect these populations to be phenotypically similarly differentiated in the wild despite lack of genetic differentiation, but this is clearly not the case (figure 1; electronic supplementary material, table S2).

(b) Implications for the mechanism of speciation

If disruptive selection initiates a speciation process, its strength is expected to be strongest when species are in the earliest stages of divergence, or actually after a population, relying on a broad resource distribution, reached carrying capacity, but before it starts to diverge [63]. Once evolutionary response to disruptive selection starts to occur, the fitness surface is expected to flatten because of the increase in phenotypic variance and the associated dissipation of disruptive selection [9,63]. Contrary to these predictions, we see in the *Pundamilia* species pairs at early stages of speciation only very weak signatures of disruptive ecological selection on morphology (Luanso, Kissenda and Python islands), but we see stronger disruptive selection in the much more advanced stage of speciation represented by the pair at Makobe Island [20,22,23] (figure 3). In addition, Meier *et al.* [62] recently found that several genomic signatures of selection also seem to be more prevalent in highly divergent regions of the genome in the *Pundamilia* of Makobe compared to the *Pundamilia* of Python. The discrepancy between observed and expected patterns could be due to differences between the way resource competition and selection are modelled in classical models of speciation by disruptive selection and the actual form of selection in nature. In classical models, disruptive selection emerges dynamically from competition for an originally unimodally distributed resource. The population depletes the most common resource which results in disruptive and negative frequency-dependent selection around the emergent dip in the resource distribution. In the studied cichlid populations, disruptive selection is likely exerted by trade-offs between functional requirements for feeding on qualitatively different types of food or for feeding in different microhabitats. Disruptive selection is likely to be imposed externally, depending on whether a population expands into the alternative niches or not, and therefore is not necessarily expected to relax as speciation proceeds. It is tempting to speculate that disruptive natural selection on morphology contributes to divergence between the *Pundamilia* species pairs mainly after speciation has reached a state where the sibling species have already evolved strong behavioural assortative mating and have little gene flow [20], allowing the evolution of ecological character displacement.

The more prevalent evidence for disruptive natural selection on morphology and ecology (figures 2b, 3), as well as for divergent evolutionary response to this selection in our *Neochromis* species pairs from Makobe and Igombe Islands, on the other hand, is consistent with speciation initiated by disruptive ecological selection. Comparing *Neochromis* and *Pundamilia* replicate form pairs with similar prevalence of disruptive selection, it appears that neutral genetic differentiation is weaker in *Neochromis*. This is consistent with the hypothesis that speciation or maintenance of species differentiation in sympatry is constrained by lack of behavioural assortative mating and facilitated when natural and sexual selection are both disruptive. Not only do the *Pundamilia* sister species differ dramatically in male nuptial coloration while the *Neochromis* pairs do not, experiments in the laboratory with *Pundamilia* revealed strong behavioural assortative mating [59], whereas equivalent experiments with the Makobe pair of *Neochromis* revealed a complete lack of behavioural assortative mating (O. Selz & O. Seehausen 2016, unpublished data). Both observations indicate that assortative mating might not be sufficient to maintain the genetic differentiation observed in this species pair and that disruptive

ecological selection might be indispensable for maintaining the species differentiation in sympatry. Consistent with this, our data show that ecological differentiation as measured by stable isotopes is also more distinct in the *Neochromis* Makobe incipient species than in the *Pundamilia* species pairs.

(c) Conclusion

Studying seven pairs of sympatric morphs, incipient and sibling species of Lake Victoria cichlid fish, we found evidence that morphological differences between species may have evolved under disruptive ecological selection. Present-day disruptive ecological selection appears to be operating on morphological traits and we see evidence for evolutionary responses to it, particularly in traits that have diverged between the species. Comparing four cases in the invertebrate feeder genus *Pundamilia* and three in the algae feeder genus *Neochromis*, we show that in both genera, the strongest reproductive isolation is seen in species pairs that exhibit the highest prevalence of disruptive selection and evolutionary response to it. Whereas some evidence for disruptive ecological selection was seen in all pairs, even those that had not begun to speciate, divergent evolutionary response to it was exclusively observed in pairs that have evolved at least some reproductive isolation, as measured with genomic markers. Variation in the prevalence of disruptive selection as well as constraints affecting evolutionary response to such selection are thus likely both contributing to variation in the occurrence of speciation in this system.

Neochromis is a genus in which few sympatric sister species are known and these differ little in male nuptial coloration. In this genus, reproductive isolation between sympatric forms became evident only when disruptive natural selection was much more prevalent than in *Pundamilia*, where sister species are more common and exhibit conspicuously different male coloration and female mating preferences based on these. This is consistent with the expectation that sympatry-robust speciation is facilitated when both natural and sexual selection are divergent. We propose that different mechanisms dominate the speciation process in the two genera, whereby selection on feeding related traits in *Pundamilia* becomes important and effective only after assortative mating has already evolved, consistent with earlier work on sexual selection and selection on the visual system [23,42,44]. In *Neochromis*, selection on feeding related traits as well as evolutionary response to it are more prevalent already at the incipient stage of speciation but genomic differentiation remains modest.

Ethics. This research was done under research permits no. 2013-256-NA-2014-177 (J.C.v.R.), 2013-251-NA-2014-177 (O.S.) and 2013-251-ER-2014-177 (F.N.M.) from the Tanzania Commission for Science and Technology (COSTECH). The growth experiment was performed under permit no. BE18/15 from the veterinary service in Bern, Switzerland.

Data accessibility. All data are available at Dryad (<http://dx.doi.org/10.5061/dryad.vk23f>) [64].

Authors' contributions. J.C.v.R. carried out fieldwork, generated morphological and stable isotope data, participated in the design of the study, carried out the statistical analyses and drafted the manuscript; F.N.M. carried out fieldwork, generated morphological data, conducted the growth experiment, performed statistical analyses, participated in the design of the study and helped drafting the manuscript; D.F. measured morphological data; O.S. designed the study, coordinated the study and wrote the manuscript. All authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

Funding. This research was supported by Swiss National Science Foundation grant no. 31003A_144046 to O.S.

Acknowledgements. We are thankful to the Tanzanian Fisheries Research Institute (TAFIRI) for their support and cooperation during the fieldwork. We thank Mhoja Kayeba, Mohamed Haluna, Jonathan Makoye, Godfrey Ngupula, Oliver Selz, Joana Meier, Shane Wright and Salome Mwaiko for their help in the field. Alois Denervaud,

Jonas Walker and Ariane le Gros are thanked for preparing stable isotope samples and for conducting morphological measurements. We thank Isabel Santos-Magalhaes for help with interpretation of morphological data. We thank the Seehausen lab and members of the Eawag Center for Ecology, Evolution and Biogeochemistry for helpful discussions and feedback at various stages of the project.

References

- Bolnick DI, Fitzpatrick BM. 2007 Sympatric speciation: models and empirical evidence. *Ann. Rev. Ecol. Evol. Syst.* **38**, 459–487. (doi:10.1146/annurev.ecolsys.38.091206.095804)
- Coyne JA, Orr HA. 2004 *Speciation*. Sunderland, MA: Sinauer Associates.
- Nosil P. 2012 *Ecological speciation*. Oxford, UK: Oxford University Press.
- Bird CE, Fernandez-Silva I, Skillings DJ, Toonen RJ. 2012 Sympatric speciation in the post 'Modern Synthesis' era of evolutionary biology. *Evol. Biol.* **39**, 158–180. (doi:10.1007/s11692-012-9183-6)
- Nosil P, Harmon LJ, Seehausen O. 2009 Ecological explanations for (incomplete) speciation. *Trends Ecol. Evol.* **24**, 145–156. (doi:10.1016/j.tree.2008.10.011)
- Smadja CM, Butlin RK. 2011 A framework for comparing processes of speciation in the presence of gene flow. *Mol. Ecol.* **20**, 5123–5140. (doi:10.1111/j.1365-294X.2011.05350.x)
- Price T. 2008 *Speciation in birds*. Greenwood Village, CO: Roberts & Company.
- Rueffler C, Van Dooren TJM, Leimar O, Abrams PA. 2006 Disruptive selection and then what? *Trends Ecol. Evol.* **21**, 238–245. (doi:10.1016/j.tree.2006.03.003)
- Bolnick DI, Doebeli M. 2003 Sexual dimorphism and adaptive speciation: two sides of the same ecological coin. *Evolution* **57**, 2433–2449. (doi:10.1111/j.0014-3820.2003.tb01489.x)
- Smith TB. 1993 Disruptive selection and the genetic-basis of bill size polymorphism in the african finch pyrenestes. *Nature* **363**, 618–620. (doi:10.1038/363618a0)
- Hori M. 1993 Frequency-dependent natural selection in the handedness of scale-eating cichlid fish. *Science* **260**, 216–219. (doi:10.1126/science.260.5105.216)
- Joron M *et al.* 2011 Chromosomal rearrangements maintain a polymorphic supergene controlling butterfly mimicry. *Nature* **477**, 203–206. (doi:10.1038/nature10341)
- Svanback R, Pineda-Krch M, Doebeli M. 2009 Fluctuating population dynamics promotes the evolution of phenotypic plasticity. *Am. Nat.* **174**, 176–189. (doi:10.1086/600112)
- Felsenstein J. 1981 Skepticism towards santa rosalia, or why are there so few kinds of animals. *Evolution* **35**, 124–138. (doi:10.1111/j.1558-5646.1981.tb04864.x)
- Gavrilets S. 2003 Perspective: models of speciation: what have we learned in 40 years? *Evolution* **57**, 2197–2215. (doi:10.1111/j.0014-3820.2003.tb00233.x)
- Kirkpatrick M, Ravigné V. 2002 Speciation by natural and sexual selection: models and experiments. *Am. Nat.* **159**, S22–S35. (doi:10.1086/338370)
- van Doorn GS, Dieckmann U, Weissing FJ. 2004 Sympatric speciation by sexual selection: a critical reevaluation. *Am. Nat.* **163**, 709–725. (doi:10.1086/383619)
- van Doorn GS, Edelaar P, Weissing FJ. 2009 On the origin of species by natural and sexual selection. *Science* **326**, 1704–1707. (doi:10.1126/science.1181661)
- Wagner CE, Harmon LJ, Seehausen O. 2014 Cichlid species-area relationships are shaped by adaptive radiations that scale with area. *Ecol. Lett.* **17**, 583–592. (doi:10.1111/Ele.12260)
- Meier JI, Sousa VC, Marques DA, Selz O, Wagner CE, Excoffier L, Seehausen O. 2016 Demographic modelling with whole-genome data reveals parallel origin of similar *Pundamilia* cichlid species after hybridization. *Mol. Ecol.* **26**, 123–141. (doi:10.1111/mec.13838)
- Malinsky M *et al.* 2015 Genomic islands of speciation separate cichlid ecomorphs in an East African crater lake. *Science* **350**, 1493–1498. (doi:10.1126/science.aac9927)
- Magalhaes IS, Lundsgaard-Hansen B, Mwaiko S, Seehausen O. 2012 Evolutionary divergence in replicate pairs of ecotypes of Lake Victoria cichlid fish. *Evol. Ecol. Res.* **14**, 381–401.
- Seehausen O *et al.* 2008 Speciation through sensory drive in cichlid fish. *Nature* **455**, 620–626. (doi:10.1038/nature07285)
- Lande R, Arnold SJ. 1983 The measurement of selection on correlated characters. *Evolution* **37**, 1210–1226. (doi:10.2307/2408842)
- Bolnick DI, Lau OL. 2008 Predictable patterns of disruptive selection in stickleback in postglacial lakes. *Am. Nat.* **172**, 1–11. (doi:10.1086/587805)
- Martin CH. 2012 Weak disruptive selection and incomplete phenotypic divergence in two classic examples of sympatric speciation: cameroon crater lake cichlids. *Am. Nat.* **180**, 90–109. (doi:10.1086/667586)
- Martin CH, Wainwright PC. 2013 Multiple fitness peaks on the adaptive landscape drive adaptive radiation in the wild. *Science* **339**, 208–211. (doi:10.1126/science.1227710)
- Schluter D. 1994 Experimental evidence that competition promotes divergence in adaptive radiation. *Science* **266**, 798–801. (doi:10.1126/science.266.5186.798)
- Schluter D. 1995 Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. *Ecology* **76**, 82–90. (doi:10.2307/1940633)
- Sogard SM. 1997 Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bull. Mar. Sci.* **60**, 1129–1157.
- Schultz ET, Clifton LM, Warner RR. 1991 Energetic constraints and size-based tactics—the adaptive significance of breeding-schedule variation in a marine fish (Embiotocidae, *Micrometrus minimus*). *Am. Nat.* **138**, 1408–1430. (doi:10.1086/285294)
- Bagenal TB. 1978 Aspects of fish fecundity. In *Ecology of freshwater fish production* (ed. SD Gerking), pp. 75–101. New York, NY: Wiley.
- Dijkstra PD, Seehausen O, Groothuis TGG. 2005 Direct male-male competition can facilitate invasion of new colour types in Lake Victoria cichlids. *Behav. Ecol. Sociobiol.* **58**, 136–143. (doi:10.1007/s00265-005-0919-5)
- Dijkstra PD, van der Zee EM, Groothuis TGG. 2008 Territory quality affects female preference in a Lake Victoria cichlid fish. *Behav. Ecol. Sociobiol.* **62**, 747–755. (doi:10.1007/s00265-007-0500-5)
- Maan ME, Seehausen O, Soderberg L, Johnson L, Ripmeester EA, Mrosso HD, Taylor MI, van Dooren TJ, van Alphen JJ. 2004 Intraspecific sexual selection on a speciation trait, male coloration, in the Lake Victoria cichlid *Pundamilia nyererei*. *Proc. R. Soc. Lond. B* **271**, 2445–2452. (doi:10.1098/rspb.2004.2911)
- Witte F, Wanink JH, Kische MA. 2007 Species distinction and the biodiversity crisis in Lake Victoria. *Trans. Am. Fish. Soc.* **136**, 1146–1159. (doi:10.1577/T05-179.1)
- Johnson TC, Scholz CA, Talbot MR, Kelts K, Ricketts RD, Ngobi G, Beuning K, Ssemmanda I, McGill JW. 1996 Late pleistocene desiccation of Lake Victoria and rapid evolution of cichlid fishes. *Science* **273**, 1091–1093. (doi:10.1126/science.273.5278.1091)
- Seehausen O. 1996 *Lake Victoria rock cichlids: taxonomy, ecology, and distribution*. Zevenhuizen, The Netherlands: Verduyn.
- Seehausen O, Schluter D. 2004 Male-male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes. *Proc. R. Soc. Lond. B* **271**, 1345–1353. (doi:10.1098/rspb.2004.2737)
- Selz OM, Thommen R, Pierotti ME, Anaya-Rojas JM, Seehausen O. 2016 Differences in male coloration are predicted by divergent sexual selection between populations of a cichlid fish. *Proc. R. Soc. B* **283**, 20150172. (doi:10.1098/rspb.2016.0172)

41. Seehausen O. 2015 Process and pattern in cichlid radiations—inferences for understanding unusually high rates of evolutionary diversification. *New Phytol.* **207**, 304–312. (doi:10.1111/nph.13450)
42. Seehausen O, Lippitsch E, Bouton N, Zwennes H. 1998 Mbipi, the rock-dwelling cichlids of Lake Victoria: description of three new genera and fifteen new species (Teleostei). *Ichthyol. Expl. Freshw.* **9**, 129–228.
43. Brawand D *et al.* 2014 The genomic substrate for adaptive radiation in African cichlid fish. *Nature* **513**, 375–381. (doi:10.1038/nature13726)
44. Seehausen O, van Alphen JJM, Witte F. 1997 Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* **277**, 1808–1811. (doi:10.1126/science.277.5333.1808)
45. Barel CDN, van Oijen MJP, Witte F, Witte-Maas ELM. 1977 Introduction to taxonomy and morphology of haplochromine cichlidae from Lake Victoria—manual to greenwoods revision papers. *Neth. J. Zool.* **27**, 333–389. (doi:10.1163/002829677X00199)
46. Vander Zanden MJ, Rasmussen JB. 1999 Primary consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the trophic position of aquatic consumers. *Ecology* **80**, 1395–1404. (doi:10.1890/0012-9658(1999)080[1395:PCCANA]2.0.CO;2)
47. Post DM. 2002 Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83**, 703–718. (doi:10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2)
48. Zanden MJV, Rasmussen JB. 2001 Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation: implications for aquatic food web studies. *Limnol. Oceanogr.* **46**, 2061–2066. (doi:10.4319/lo.2001.46.8.2061)
49. Cheung CHY, Chaille PM, Randall DJ, Gray JS, Au DWT. 2007 The use of scale increment as a means of indicating fish growth and growth impairment. *Aquaculture* **266**, 102–111. (doi:10.1016/j.aquaculture.2007.02.036)
50. Doyle RW, Talbot AJ, Nicholas RR. 1987 Statistical interrelation of length, growth, and scale circulus spacing—appraisal of a growth-rate estimator for fish. *Can. J. Fish. Aquat. Sci.* **44**, 1520–1528. (doi:10.1139/F87-183)
51. Kingsford MJ, Atkinson MH. 1994 Increments in otoliths and scales—how they relate to the Age and early development of reared and wild larval and juvenile *Pagrus auratus* (Sparidae). *Aust. J. Mar. Freshw. Res.* **45**, 1007–1021. (doi:10.1071/MF9941007)
52. Stinchcombe JR, Agrawal AF, Hohenlohe PA, Arnold SJ, Blows MW. 2008 Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? *Evolution* **62**, 2435–2440. (doi:10.1111/j.1558-5646.2008.00449.x)
53. Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hill CE, Hoang A, Gibert P, Beerli P. 2001 The strength of phenotypic selection in natural populations. *Am. Nat.* **157**, 245–261. (doi:10.1086/319193)
54. Chenoweth SF, Hunt J, Rundle HD. 2012 Analyzing and comparing the geometry of individual fitness surfaces. In *The adaptive landscape in evolutionary biology* (eds E Svensson, R Calsbeek), pp. 126–149. Oxford, UK: Oxford University Press.
55. R Core Team. 2015 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
56. Blows MW, Brooks R. 2003 Measuring nonlinear selection. *Am. Nat.* **162**, 815–820. (doi:10.1086/378905)
57. Schluter D, Nychka D. 1994 Exploring fitness surfaces. *Am. Nat.* **143**, 597–616. (doi:10.1086/285622)
58. Friedman JH, Stuetzle W. 1981 Projection pursuit regression. *J. Am. Stat. Assoc.* **76**, 817–823. (doi:10.2307/2287576)
59. Selz OM, Pierotti MER, Maan ME, Schmid C, Seehausen O. 2014 Female preference for male color is necessary and sufficient for assortative mating in 2 cichlid sister species. *Behav. Ecol.* **25**, 612–626. (doi:10.1093/beheco/aru024)
60. Stauffer JR, Van Snik Gray EV. 2004 Phenotypic plasticity: its role in trophic radiation and explosive speciation in cichlids (*Teleostei : Cichlidae*). *Anim. Biol.* **54**, 137–158. (doi:10.1163/1570756041445191)
61. Magalhaes IS, Mwaiko S, Schneider MV, Seehausen O. 2009 Divergent selection and phenotypic plasticity during incipient speciation in Lake Victoria cichlid fish. *J. Evol. Biol.* **22**, 260–274. (doi:10.1111/j.1420-9101.2008.01637.x)
62. Meier JI, Marques DA, Wagner CE, Excoffier L, Seehausen O. Submitted. Genomics of parallel ecological speciation in Lake Victoria cichlids. *Mol. Biol. Evol.*
63. Dieckmann U., Doebeli M. 1999 On the origin of species by sympatric speciation. *Nature* **400**, 354–357. (doi:10.1038/22521)
64. van Rijssel JC, Moser FN, Frei D, Seehausen O. 2018 Prevalence of disruptive selection predicts extent of species differentiation in Lake Victoria cichlids. Dryad Digital Repository. (<http://dx.doi.org/10.5061/dryad.vk23f>)