Evolution, 2023, **77(3)**, 823–835
https://doi.org/10.1093/evolut/qpad001
Advance access publication 7 January 2023
Original Article

Constraints to speciation despite divergence in an old haplochromine cichlid lineage

Claudio Müller[1,2,†], Florian N. Moser[1,2,†], David Frei[1,2], Ole Seehausen[1,2]

[1]Department of Aquatic Ecology, Institute of Ecology and Evolution, University of Bern, 3012 Bern, Switzerland
[2]Department of Fish Ecology & Evolution, EAWAG, Centre for Ecology, Evolution and Biogeochemistry, 6047 Kastanienbaum, Switzerland
Corresponding author: SL B16, Ole Seehausen, Eawag Seestrasse 79, 6047 Kastanienbaum, Switzerland. Email: ole.seehausen@eawag.ch
†Shared first author.

Abstract

Most of the 500+ cichlid species of Lake Victoria evolved very rapidly in the wake of an adaptive radiation within the last 15,000 years. All 500 species have evolved from just one out of five old cichlid lineages that colonized the lake. Endemic to the Lake Victoria region, *Astatoreochromis alluaudi* is a member of an old haplochromine lineage that never speciated in the region. Even though the species occurs in a wide range of habitats, there were no indications of evolutionary diversification. Here, we tested predictions of several hypothetical mechanisms that might constrain speciation, including high dispersal rates, a generalist life style and the lack of behavioral assortative mating. Genomic analyses of individuals from 13 populations revealed several genomically distinct groups, associated with major habitat classes, indicating the existence of two distinct ecotypes. We found significant phenotypic differences between these ecotypes in the wild, which were retained under common-garden conditions, potentially indicating heritable phenotypic adaptations. Female mate choice experiments revealed the absence of behavioral assortative mating despite genetic and phenotypic differentiation between ecotypes. We suggest that the lack of coupling between behavioral mating preferences and phenotypic and genetic divergence constrains speciation in this cichlid.

Keywords: adaptive radiation, cichlid fish, mate choice, population genomics, reproductive isolation, speciation

Introduction

There is a large variation in species richness and speciation rates between different taxonomic lineages (Jablonski, 2008; Schluter & Pennell, 2017; Weir & Schluter, 2007). While some of the factors associated with differences in species richness and speciation rates have been identified (Grant & Grant, 2008; Hugall & Stuart-Fox, 2012; Rabosky, 2016; Rolland et al., 2014), a lot of the observed variation remains unexplained. Many of the species-rich clades show evidence of adaptive radiations; instances where few founder species rapidly diversify into numerous species adapted to different ecological niches (Schluter 2000). Interestingly, many members of well studied adaptive radiations co-occur with related lineages that did not radiate and remained species poor. For instance, numerous species of Darwin finches co-occur with a single species of mockingbird on each of the Galapagos Islands (Arbogast et al., 2006), and the Caribbean Anoles co-occur with several other lizard clades that did not diversify (Crother & Guyer, 1996; Losos, 2009). Such settings where lineages with high and lineages with low speciation rates co-occur in a narrow geographic range provide ideal opportunities to study the mechanisms associated with speciation and species persistence.

African cichlids are a famous study system for investigating adaptive radiation (Kocher, 2004; Ronco et al., 2020; Seehausen, 2006; Turner, 2007) because they radiated in multiple lakes into high numbers of different species (Genner et al., 2004; Turner et al., 2001; Wagner et al., 2014). The evolutionary young age of some of these radiations and the large variety of ecological niches occupied by the different cichlid species in these lakes, make them an excellent system to study speciation and adaptation (Seehausen, 2006).

The youngest large radiation in the East African Lakes can be found in Lake Victoria, where more than 500 haplochromine cichlid species evolved in less than 15,000 years (Johnson et al., 1996), showing an exceptionally high speciation rate for vertebrates (Rabosky et al., 2013; Seehausen, 2002, 2015). However, not all cichlid lineages that colonized Lake Victoria diversified equally quickly. In fact, only one of the five cichlid lineages that colonized Lake Victoria accounts for most of the species richness within the lake (Seehausen, 2002, 2006; Wagner et al., 2012).

The haplochromine cichlid genus *Astatoreochromis* is widespread across several rivers and large lakes and in many little lakes in East Africa, occupying a large variety of different habitats. However, there are only two species described in the genus (Banyankimbona et al., 2013): *Astatoreochromis straeleni*, which is restricted to Lake Tanganyika and surrounding rivers, and *A. alluaudi*, which occurs in the basins of Lake Victoria and Lake Edward. The latter is a generalist (Binning & Chapman, 2008; Witte, 1981) and can be found in many habitats, some of which have facilitated very high rates of speciation in other haplochromine cichlids. In Lake Victoria it is mainly molluscivorous (Greenwood, 1965; Hoogerhoud, 1986; Mbabazi, 2004; Witte, 1981), but its diet can vary drastically depending on the environment and food

Received September 21, 2022; revisions received December 19, 2022; accepted January 6, 2023

availability. *Astatoreochromis alluaudi* is well known for its phenotypic plasticity, especially in the lower pharyngeal jaw, expressed in response to different food sources (Huysseune, 1995; Smits et al., 1996; Witte & Van Oijen, 1990; Young, 2013). Phenotypic plasticity can hamper or facilitate speciation. On one hand, it can hamper evolutionary divergence by acting as a buffer against divergent selection (e.g., Agrawal, 2001). On the other hand, it can be key to facilitate speciation and adaptive radiation for instance by allowing rapid ecological range expansion or by enhancing phenotypic differentiation and adaptive habitat choice (Nonaka et al., 2015; Pfennig et al., 2006, 2010; Schneider & Meyer, 2017).

In this study we investigated genetic and ecological divergence among 13 populations of *A. alluaudi,* most of them from Lake Victoria but also from Lake Burungi and Lake Rwakajunju in the Kagera River region upstream of Lake Victoria and Lake Saka in the Lake Edward catchment, to the West of Lake Victoria (Figure 1). We chose populations to represent fish from two contrasting habitats: rocky mainland and island reefs in the relatively clear waters of the open lake on the one hand, and on the other hand vegetated swamps, mainland shores and inshore islands in Lake Victoria and satellite lakes (Figure 2). Further, we investigated the phenotypic divergence between two populations from ecologically distinct habitats, performed a common garden rearing experiment and experimentally measured behavioral reproductive isolation between these two focal populations. One of these populations is from a swampy, vegetation-rich, slow-flowing inlet stream entering the Mwanza Gulf at the village Sweya in Nyegezi Bay. The other population is from Makobe Island, an offshore rocky reef composed of bare rocks and hardly any aquatic vegetation (Figure 2). Makobe Island hosts a very species-rich cichlid community with around 30 distinct species of the haplochromine radiation as well as some non-radiated *Oreochromis* species (Seehausen & Bouton, 1997). At Makobe Island many radiation genera occur in two or more closely related species that often occupy slightly different water depth ranges, or differ in the degree to which they live inside of crevices or outside in the open, and are often ecologically distinct in their feeding as well (Bouton et al., 1997; Seehausen & Bouton, 1997; Seehausen et al., 2008). *Astatoreochromis alluaudi* occurs at the exact same location where it occupies the entire available depth range, and occurs in crevices as well as outside, overlapping in habitat utilization with all these other species. Yet, it shows no evidence for morphological or genetic divergence between the deep- and the shallow-living individuals (Moser, 2018, unpublished).

The goal of this study is to better understand the factors that constrain speciation in *A. alluaudi*. In this study we investigated three potential factors. First, we investigated whether divergence within *A. alluaudi* may be hampered due to high dispersal rates, which may prevent genetic divergence between populations and parapatric or allopatric speciation. Second, we tested whether *A. alluaudi* is a habitat generalist that does not require heritable adaptation to different environments since the species would be equally well suited to most habitats it occupies. This would mean that either phenotypes are similarly well equipped to thrive in different habitats, or individuals adapt to the environment by means of adaptive phenotypic plasticity. In either case no strong divergent selection and therefore no heritable phenotypic divergence would be expected. Third, we investigated whether differences in ecologically, genetically and phenotypically distinct allopatric populations may not have any effects on behavioral mating preferences and hence reproductive isolation, which could otherwise maintain differentiation in secondary contact. Reproductive isolation that is maintained in contact is a crucial step in the speciation process in cichlids (Feller et al., 2020; Seehausen, 2009).

If distinct populations of *A. alluaudi* are genetically and phenotypically differentiated and can retain their distinctiveness in sympatry, the premise of a lack of speciation in this lineage might have to be reevaluated.

Material and methods

Fish sampling

Astatoreochromis alluaudi were caught from 13 locations in the south of Lake Victoria, in Lake Burigi, Lake Rwakajunju,

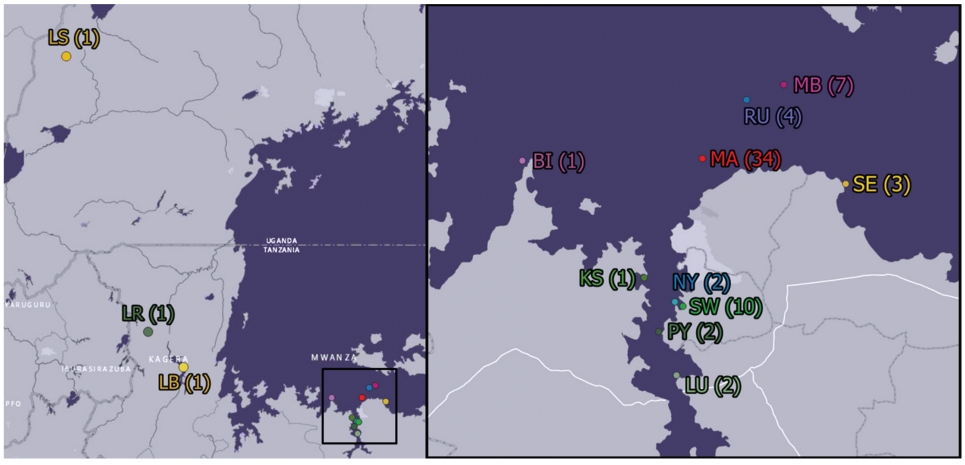

Figure 1. Map of sampling sites for *Astatoreochromis alluaudi* used in this study. We sampled from three more offshore rocky islands in Lake Victoria, Makobe Island (MA), Ruti Island (RU) and Mabibi Island (MB), two mainland sites or nearshore islands in the open lake, Bihiru (BI) and Senga (SE) and from five populations within the Mwanza Gulf: Kissenda (KS) in the NW of the Gulf, Sweya (SW), Nyegezi (NY), Python (PY) and Luanso (LU) in the East. Additionally, we included two individuals from lakes west (and upstream) of Lake Victoria (Lake Burigi and Lake Rwakajunju) and the published sequence of an individual from Lake Saka (LS) in the Lake Edward drainage. The numbers in brackets show the number of individuals per location used for the genomics analyses.

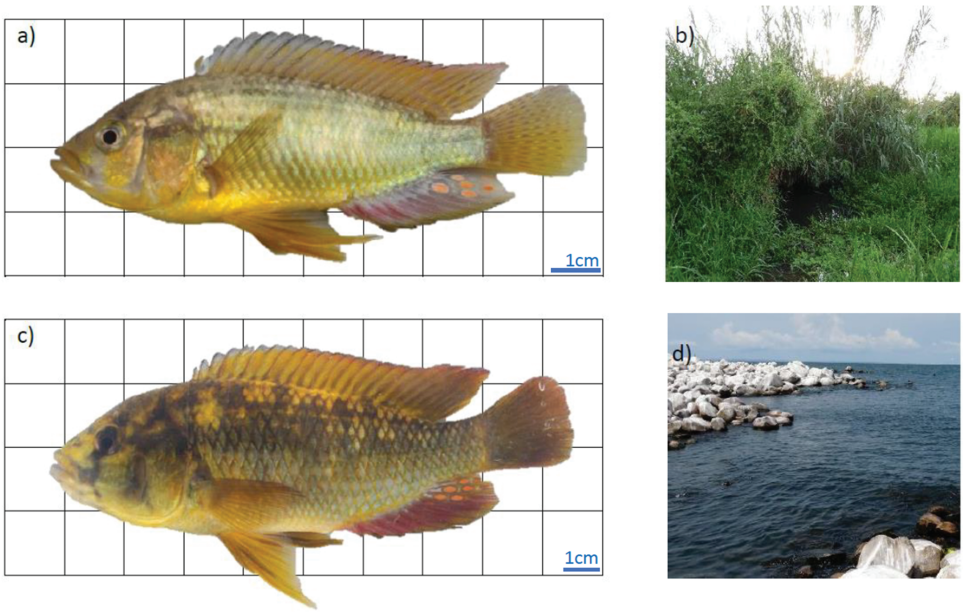

Figure 2. A wild-caught male *Astatoreochromis alluaudi* collected from Sweya (a), a vegetation-rich inlet stream (b) and a wild-caught male *A. alluaudi* caught at Makobe Island (c), an offshore rocky island (d).

and Lake Saka (Figure 1) by angling, gill netting and minnow traps in several field seasons between 2000 and 2014. From all fish, standardized live pictures were taken before the fish were euthanized with an overdose of Phenoxyethanol. Of every fish, the pelvic fin of the right side was clipped and stored in 100% analytical ethanol for DNA extraction. Voucher specimens were fixed in 4% formalin, rinsed in water and transferred to ethanol through a 3-step dilution series before they were stored in 75% ethanol at the EAWAG Center for Ecology, Evolution and Biogeochemistry (CEEB) in Kastanienbaum, Switzerland.

Additionally, 15 adult individuals from Sweya and 18 adult individuals from Makobe were collected and transported alive to the aquarium facilities at the CEEB in 2014 for common garden rearing and mate choice experiments. To produce lab-bred individuals, fish from the wild were kept in population-specific tanks, where they could interact and mate freely. We obtained six F1 clutches from the Makobe population and 10 F1 clutches from the Sweya population. These were raised separately (one clutch per tank) in identical 20 cm/40 cm/20 cm (length/depth/height) tanks. When they reached a standard length (SL) of more than 15 mm, the clutches were consecutively transferred into larger tanks. As soon as male offspring started to express sex-specific coloration, they were separated from their siblings and raised further in clutch-specific male-only tanks. This was done to prevent any experience with mate choice and courtship prior to our experiments. All tanks had a gravel floor, a 12 hr light–dark cycle, were connected to the same flow-through system and fish were fed flake food ad libitum on a daily basis and a mix of peas and shrimps once a week.

Genomics

DNA extraction and sequencing

To investigate population genomic patterns, we used genome-wide restriction site-associated DNA (RAD) sequencing. We sequenced seven fish from Mabibi Island, four from Ruti Island, 34 fish from Makobe Island, one individual from Bihiru, three individuals from the rocky mainland shores of Senga, one individual from Kissenda Island from the western shore of the Mwanza Gulf, as well as 10 individuals from Sweya and two individuals from Nyegezi, from Python and from Luanso each, and one individual from Lake Burigi and Lake Rwakajunju (Figure 1). Individuals from Makobe Island and Sweya were caught by targeted sampling for this study in the field season 2014, while the individuals from other sites were collected while sampling for other species in other years, resulting in smaller numbers. Additionally, we used the published sequence from one individual from Lake Saka in the Lake Edward catchment (Figure 1; Meier et al., 2017a). We extracted DNA from fin clips using the phenol chloroform DNA extraction protocol provided by Sambrook and Russell (2001), and prepared RAD-libraries using the protocol by Baird et al. (2008) with minor changes indicated in Moser et al. (2018). Sequencing was conducted on an Illumina HiSeq2500 platform at the Centre of Integrative Genomics, University of Lausanne.

Population genomic and phylogenomic analyses

We filtered the raw reads for high quality reads with an intact *Sbf*I restriction site, and de-multiplexed and barcode trimmed those, using the FASTX toolkit v.0.0.13 (Gordon & Hannon, 2010) and custom-made Python scripts. These reads were mapped against the reference genome of *Oreochromis niloticus* (Brawand et al., 2014) with Bowtie2 version 1.0.1 (Langmead & Salzberg, 2012) and default end-to-end alignment parameters. We used empirical error rate estimations from bacteriophage PhiX reads for base quality score recalibration (Marques et al., 2016). Next, we called genotypes and variants with the GATK tool UnifiedGenotyper (McKenna et al., 2010), and filtered the resulting vcf file for genotype depth (>3), genotype quality (>30) and missing data (<20% per site) and removed indels with vcftools (Danecek et al., 2011). Further we removed individuals with more than 75% missing

data. RAxML v8.0.0 (Stamatakis, 2014) was used to build a maximum likelihood tree using all concatenated sequences (~1.69 Mio reads) from individuals from all sampling sites. For each of 100 bootstrap replicates, we resampled sites from the concatenated dataset. From each resampled dataset, the maximum likelihood tree was inferred using a GTRGAMMA model. Bootstrap support was then calculated based on these 100 topologies and was plotted on the edges of the best-scoring maximum likelihood tree calculated from the complete data set.

For principal component analysis (PCA), we removed all monomorphic sites, used only SNPs with a genotype depth >20, genotype quality >30 and less than 20% missing data, and applied a 5% minor allele cutoff in vcftools. Individuals with more than 45% missing data were excluded. PCA was performed in R, using the packages gdsfmt and SNPRelate (Zheng et al., 2012).

For STRUCTURE analysis (Pritchard et al., 2000) we removed all monomorphic sites and indels, used only SNPs with a genotype depth >10, genotype quality >30 and less than 20% missing data, and applied a 5% minor allele frequency cutoff in vcftools. Additionally, we excluded individuals with more than 60% missing data and individuals with highly unbalanced allele frequencies at heterozygous sites, to reduce the effect of potential PCR-errors. We ran STRUCTURE for K = 1–6 with six runs for every K. The best supported number of clusters was evaluated with StructureHarvester (Earl & Vonholdt, 2012).

To test for isolation by distance (IBD) and isolation by ecology (IBE), we calculated identity by state (IBS) between all individuals. IBS was calculated using the same dataset that we used to calculate the PCA, but we retained individuals with up to 80% missing data. IBS was then calculated in R, using the Package SNPrelate (Zheng et al., 2012). To get a measure of relatedness between populations, we calculated the average of each individual pairing between two populations (Supplementary Table S1). To test for IBD, we constructed a distance matrix with the geographical distances between the different populations using google maps (Supplementary Table S2). To test for IBE, we used five ecological variables: vegetation (categorical with three states: none, some, plenty), substrate (binary, rock or mud), turbidity (continuous, Secchi-depth), depth range (continuous, maximal sampled water depth at the sampling location in m), and distance to the mainland shore (continuous, in km; Supplementary Table S3). To estimate the ecological distance (habitat contrast) between any two sampling sites, we performed a PCA in R, including all these five ecological and environmental variables. The distance between two populations in the ecospace spanned by PC1 (explains 70.00% of variance) and PC2 (explains 16.96% of variance) was used as an estimate of the ecological habitat contrast between the two populations (Supplementary Table S4). To calculate effects consistent with IBD and IBE, we conducted Mantel tests in the R-package ecodist.

To test for genetic divergence between our two focal populations from Makobe Island and Sweya, we calculated F-statistics using a locus-by-locus AMOVA in Arlequin (Excoffier et al., 2005). For this, we used a stringent missing data per site cutoff (<0.8), a minor allele frequency cutoff of 5% and removed monomorphic and multiallelic SNPs.

Morphology

To quantify phenotypic variation between the *A. alluaudi* populations from Makobe Island and Sweya, representing the two major ecotypes (see Results), we measured 14 morphometric distances that have been shown to be powerful for capturing taxonomic and ecomorphological differences between closely related haplochromine cichlid species (Banyankimbona et al., 2013; Barel et al., 1977; Parsons et al., 2003). We used digital calipers to measure standard length (SL), head length (HL), head width (HW), body depth (BD), lower jaw length (LJL) and width (LJW), interorbital width (IOW), preorbital width (POW), snout width (SnW), snout length (SnL), eye length (EyL), eye depth (EyD), cheek depth (ChD) and preorbital depth (POD; figure S1) in 83 fish from our two focal populations (N = 16 for Sweya, N = 67 for Makobe Island; Figure 2). Additionally, we measured the length of all fins. Fin length provides additional information, as it can be correlated with swimming performance (Plaut, 2000; Qu et al., 2013), physical health (Bakker & Mundwiler, 1999), and mating success (Bischoff et al., 1985). Finally, we counted the number of anal fin spines, a highly conserved meristic trait used in cichlid fish systematics, which was also used to discriminate between the two described *Astatoreochromis* species (Banyankimbona et al., 2013).

To investigate phenotypic plasticity as a possible cause of morphological differences, we took standardized pictures of 30 males raised to maturity in a common-garden environment (16 Sweya, 14 Makobe) and set 7 landmarks in the program tpsDig232 (Rohlf, 2015; Supplementary Figure S2). These images of live fish were used to measure four traits corresponding to four traits measured also in the wild-caught fish: Standard length, dorsal fin length, causal fin length and anal fin length. The other traits we had measured in the wild-caught fish were not measured in our common garden laboratory fish because they would need to be measured from preserved fish and we needed to keep our fish alive for mate choice experiments (see below).

For all analyses, all morphological traits (13 body measurements and five fin measurements), except the anal fin spine counts, were log-transformed. For size-correction we ran an ANCOVA with population and the log-transformed SL as covariates for each log-transformed morphometric trait. Then we used the standardized residuals of each trait after extracting the effects of the coefficient of the linear relationship between the trait and SL to conduct t-tests for significant differences. First, we tested for differences between the two sexes of the same population, to check for sexual dimorphism, then between the two populations (Makobe and Sweya) to test for differences between the populations and finally between the two populations for each sex separately. For the anal fin spine number, we conducted a chi-square test of independence to test if the number of spines (4, 5 or 6) was independent of the population. Finally, to estimate overall morphological differentiation, we performed PCA on the 18 linear morphological traits. All analyses, if not stated differently, were performed in R (R-Core-Team, 2015).

Trophic ecology

To evaluate potential differences in trophic ecology between the two populations, we compared the stomach contents of wild-caught *A. alluaudi* individuals of Makobe Island to those from Sweya. For this purpose, we dissected the stomachs of 36 Makobe and 17 Sweya individuals and quantified volumetric proportions of the different prey categories. Items belonging to four categories (snails, mussels, shrimps and fish) could be identified in the stomachs of our fish. Unidentifiable material was summarized in a fifth category (Hyslop, 1980).

Table 1. Primers and their sequences used for the parentage analysis, with the repeat motifs and the published size ranges of the microsatellites (Sanetra et al., 2009; Taylor et al., 2002).

Primer	Sequence forward	Sequence reverse	Repeat	Size range
Ppun7	5′-TGACCATCTGCGACAAATAAC-3′	5′-AGGCCTAAGTCCCCCTAACC-3′	GATA	189–299
Ppun21	5′-GGTTGACAGCTGCAAAAATG-3′	5′-AGGCAGTGACCTCTGCTCTC-3′	GATA	311–389
OSU19T	5′-TGAAGGACAAAGCAGGACTG-3′	5′-TGCCCGAACCTTTTTATTTA-3′	CA	126–142
OSU20D	5′-GAAGTGGGATTTGCAGCTTG-3′	5′-CATGCTTACAAAGAACAGGGTTAC-3′	GT	155–211
OSU13d	5′-TAAGCTGATAGGAACCCAAC-3′	5′-ACTCCTATTTTGTTATTTTTGTGA-3′	GT	105–151

Zooplankton, detritus, insects and insect larvae, prey typically of many other haplochromines from the same habitats, were not found in any of the stomachs. From the stomach contents, we calculated Schoener's percent resource overlap index (Schoener, 1970) between *A. alluadi* from Sweya and Makobe. Stomachs that were empty (estimated fullness <25%, $N = 7$ for Sweya and $N = 2$ for Makobe) were removed from subsequent analysis.

Mate choice

For the mate choice experiment, we used naive lab-bred common garden fish to exclude environmental factors as well as any previous experience with courtship and mate choice. We conducted the experiment in 143 cm/77 cm/32.5 cm (length/depth/height) tanks, laid out with gravel. All tanks had a slow, but constant waterflow. Within the experimental tank, we used plastic mesh to create four semi-circular areas that were separated from the rest of the tank (Supplementary Figure S3). The mesh size (1.5 cm in the diagonal) was chosen to allow the females to enter and leave these fenced areas but keeping the slightly larger males from leaving their territories. This set-up prevented direct male–male interference while allowing females to directly interact with the males and mate, reducing the influence of male–male interactions on female mate choice to a minimum.

The evening before a trial started, we placed four males, two of each population (of two different clutches each), into the four compartments within the experimental tanks. This allowed them to interact visually but not physically to animate them to express nuptial coloration. Males of the same population were always placed diagonally to each other (Supplementary Figure S3). The position of the first placed male was randomized for each trial. The trial started with the introduction of females into the experimental tank in the subsequent morning. For each trial, we used females of a single family, assuring that none of the stimulus males was full-sibling to the focal females. We used one to seven females per trial, depending on how many females we had raised per clutch. The trials lasted until one of the females had successfully mated.

After each replicate, we took a standardized picture of every male in the trial to measure their SL and fin lengths (Supplementary Figure S2). After anesthesia with a low dose of MS-222, we took a fin clip and measured their wet weight, before placing them into separate smaller individual tanks to recover. *Astatoreochromis alluaudi* are female mouth brooders. The females were retained in the experimental tank for an additional week after the males had been removed to minimize the stress for the mouth-brooding female which otherwise may lead to termination of a brood. We carefully collected all the eggs from the brooding female and incubated them for an additional week, to assure enough tissue for parentage analyses. Embryos were euthanized with an overdose of MS-222 and stored individually in 100% analytical ethanol until DNA extraction. The females that had not mated during the experiment were put back into their original family-specific female-only tank. The female that had mated was anesthetized in a 50 mg/L MS-222 solution to take a fin clip for genetic parentage analysis and was then put into a separate tank for recovery.

For parentage analysis, we used tissue from the fin clips of the parents and tissues from the caudal fins of the embryos. We extracted the DNA following the Chelex extraction protocol. We prepared a primer mix, containing 1.5 µl of each primer (Table 1; Sanetra et al., 2009; Taylor et al., 2002) and 55 µl TE-Buffer. For each individual we mixed 0.75 µl of the extracted DNA solution, with 4.25 µl Master-Mix (57% Multiplex Kit, 40% deionized water, 3% Primer Mix) to conduct a PCR (Supplementary Table S5). Genotyping at the five microsatellite loci was performed on an ABI 3130xl Genetic Analyzer (DNA CoreLab) with Size Standard LIZ600. Among the experimental males, we found a total of 58 alleles, of which 11 were present in both populations, 16 were unique to individuals from Sweya, and 31 were unique to individuals from Makobe Island (Supplementary Table S6).

To test for deviation from random mating, we used a General Linear Model with the mating status per replicate trial (assortative/disassortative), the father's weight ranking (R1 = heaviest, R4 = lightest) and the fathers compartment position in the experimental tank (C1 = front left, C2 = front right, C3 = back left, C4 = back right) as independent variables (Supplementary Table S11). We also conducted a *t*-test using the number of fertilized eggs from each replicate to test if the number of fertilized eggs differed between assortative and disassortative mating events.

Results

Genetic differentiation between allo-parapatric populations of *A. alluaudi*

Phylogenetic analyses based on approximately 1.69 million concatenated sites in individuals from 13 sampling sites revealed a deep split into two distinct clades (Figure 3a, bootstrap support = 100%). The first well-supported clade contains all individuals from sampling sites at the eastern shore of the Mwanza Gulf (SW, NY, PY, LU) and the individuals from the geographically distant Lakes Burigi, Rwakajunju and Saka. Within this clade, the individuals from the distant lakes form a highly supported monophyletic group (Bootstrap support = 94%). The second clade contains all fish from all rocky mainland reefs and islands in the open Lake Victoria studied here (Bihiru, Senga, Makobe, Mabibi, and Ruti) and

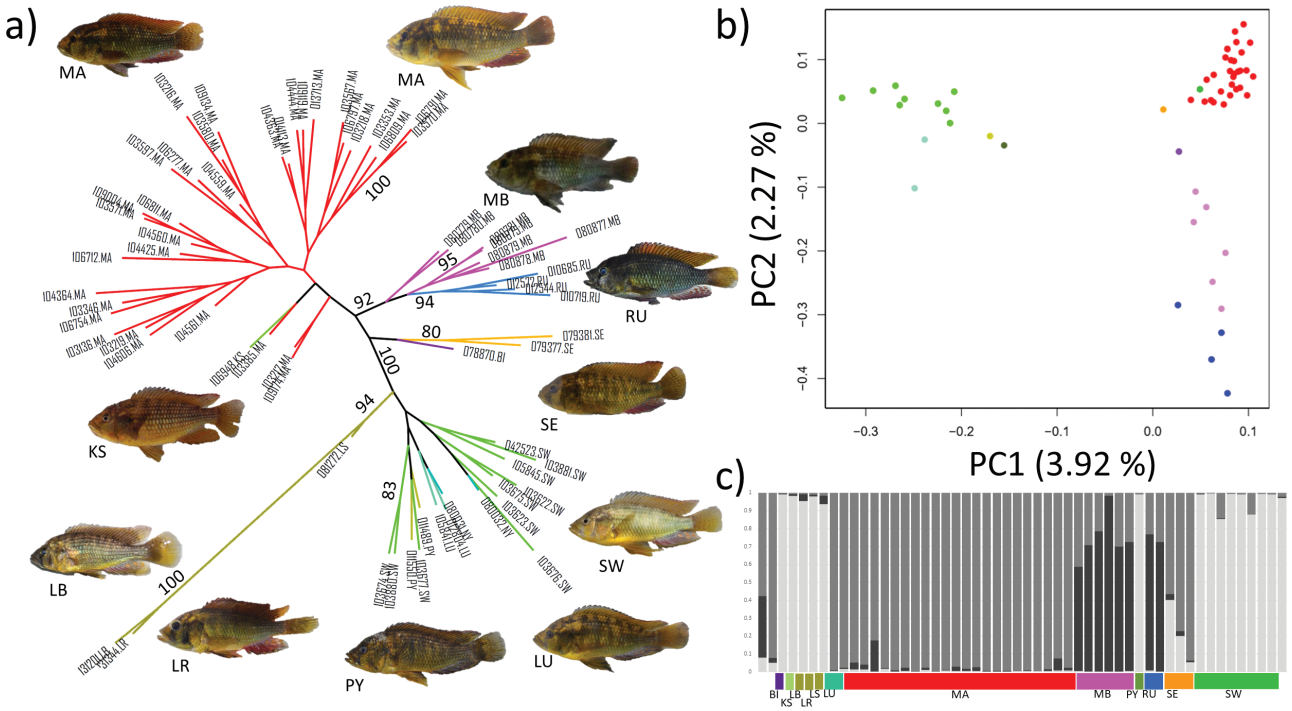

Figure 3. (a) Maximum likelihood phylogenetic tree of *Astatoreochromis alluaudi* populations based on over 2 million concatenated sites obtained by RAD sequencing reveals two well-supported clades: Eastern Mwanza Gulf (Sweya = SW, Nyegezi = NY, Python = PY, Luanso = LU; green) plus the lakes west of Lake Victoria (Lake Saka = LS, Lake Rwakajunju = LR, Lake Burigi = LB) on one hand, and the offshore rocky islands Mabibi (MB, purple) and Ruti (RU, blue) on the other hand. Makobe Island (MA, red) and Kissenda Island (KS, green) form a third clade, which is less strongly supported (13% BS). The open lake mainland reef population Senga (SE, violet) and nearshore island population Bihiru (BI, orange) fall outside those three clades. Bootstrap support higher than 70% are reported. (b) Principal component analyses based on 2,580 SNPs shows a similar pattern. Whereas PC1 separates the fish from the Eastern Mwanza Gulf and the western lakes from the rocky islands and reefs of the open lake, PC2 separates Ruti and Mabibi from Makobe, Kissenda, Senga and Bihiru. (c) STRUCTURE assignments of individuals to genetic populations. Analysis with $K = 3$ reveals a separation into (i) a population from offshore islands in the Speke Gulf (MB, RU, dark grey); (ii) a population from Makobe (MA) Island, Kissenda (KS) Island, Senga (SE) and Bihiru Island (BI; grey); (iii) a population from all eastern Mwanza Gulf sites (SW, NY, PY, LU) and the distant lakes (LB, LR, LS, light grey). Note signals of introgression between two or all three clades, especially in individuals from the mainland and nearshore reefs outside the Mwanza Gulf (SE, BI).

the individual from a rocky island near the western shore of the Mwanza Gulf (Kissenda). Within this clade, Mabibi Island and Ruti Island, the two most offshore rocky islands in the Speke Gulf, together make a well-supported group (BS = 92%). Within it, all individuals from Ruti form a well-supported monophyletic clade (BS = 94%) that is nested within a paraphyletic Mabibi clade, indicating geographic structure between the offshore rocky islands and between them and the more nearshore and inshore sites. The positions of the individuals from our two open lake mainland reef and nearshore island sites (Senga and Bihiru, respectively), relative to the others are not well resolved even though Senga might form a monophyletic clade on its own (Figure 3a). On the other hand, Makobe Island is not a strongly supported clade and our fish from Kissenda Island resides within the Makobe clade.

A PCA conducted with 2,580 SNPs derived from the RAD sequences of 54 individuals from 10 locations clearly separates the individuals from the open lake mainland reefs and islands (Mabibi, Ruti, Makobe, Senga, Bihiru) and Kissenda from all individuals from the eastern shore of the Mwanza Gulf (Sweya, Python and Luanso) and the individual from Lake Burigi on PC1, which explains 3.92% of the variance. PC2 (2.27%) separates the fish from Ruti and Mabibi Island from those of Makobe, Senga, Bihiru and Kissenda, as well as all others (Figure 3b).

STRUCTURE analyses based on 1,277 SNPs revealed a best fit for three genetic clusters ($K = 3$; Figure 3c). The first split, revealed by $K = 2$, separates all individuals from the eastern Mwanza Gulf sites (SW, PY and LU) together with Lake Saka (LS), Lake Burigi (LB) and Lake Rwakajunju (LR) from those from the mainland reefs and islands in the open lake (MA, MB, RU, BI, SE) and the northwestern Mwanza Gulf (KS; Supplementary Figure S4). Best supported $K = 3$ further separates the fish from both offshore reefs MB and RU from the other representatives of the second cluster, with about 30%–50% assignment to cluster 2. The two open lake mainland and nearshore reef populations (SE and BI) mainly were assigned to the Makobe cluster; but there are indications of introgression from the offshore island cluster into BI and from the eastern Mwanza Gulf cluster into SE. This confirms the pattern revealed by the genomic PCA with the clearest separation between the individuals from the eastern Mwanza Gulf, LB, LR, and LS, and the other locations (along PC1 and in $K = 2$), and an additional split, between Makobe and Kissenda and the two offshore rocky reef populations from the Speke Gulf (RU and MB).

Genetic differentiation is mainly explained by habitat contrast

The Mantel tests revealed a highly significant correlation between genetic distance and ecological distance (permutations = 10,000, $r_m = 0.416$, $p = .003$), but no correlation

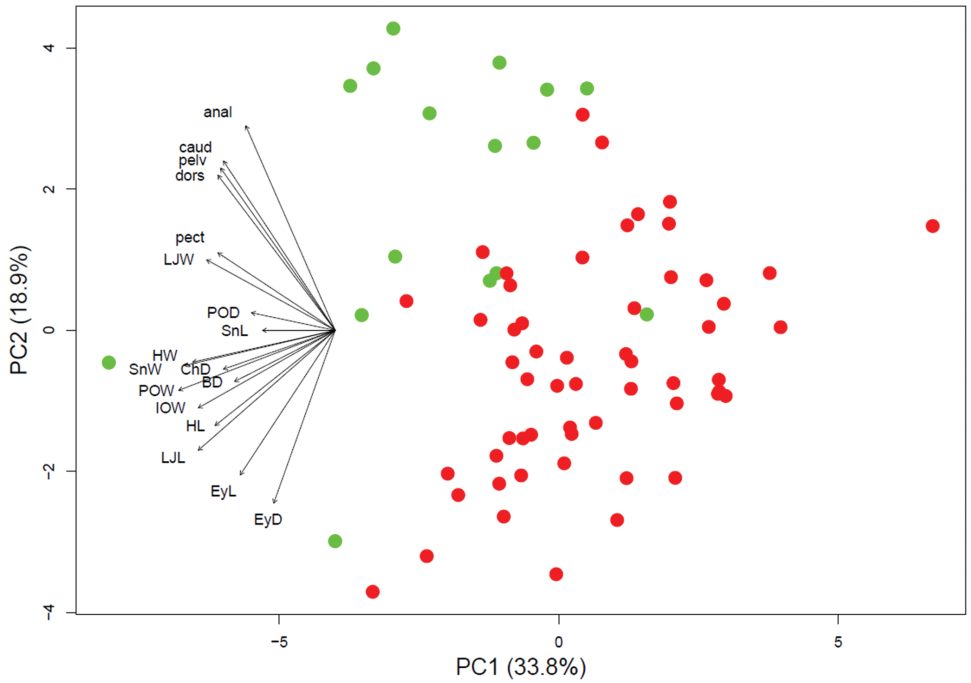

Figure 4. Principal component analysis of linear morphometric traits of *Astatoreochromis alluaudi* from a reef ecotype population (Makobe, red) and a vegetation ecotype population (Sweya, green). It reveals phenotypic differences between the populations with Sweya individuals having a wider head (negative PC1) longer fins and smaller eyes (positive PC2) than individuals from Makobe. The black arrows on the left indicate the loadings of the different morphological traits with the different fin lengths in small letters and the other morphological traits in capital letters.

between genetic and geographic distance (permutations = 10,000, $r_m = -0.145$, $p = .374$). Using partial Mantel tests to test for IBE after correcting for geographic distance resulted in a highly significant pattern of IBE (permutations = 10,000, $r_m = 0.507$, $p < .001$), whereas IBD after correcting for ecological distance is significant too but with a much higher p-value (permutations = 10,000, $r_m = 0.348$, $p = 0.033$). The genetic structure among *Astatoreochromis* populations therefore is mainly explained by ecological differences between habitats, with only a weak IBD.

The two distinct genetic clusters that we found along PC1 and in STRUCTURE with $K = 2$, correspond to the two habitat types with very distinct ecological characteristics. One of the two clusters contains all individuals from the three rocky islands in the open lake (Makobe, Ruti and Mabibi) and also the rocky nearshore island (Bihiru) and mainland shore outside the Mwanza Gulf (Senga) but occurs also at Kissenda Island in the northwestern Mwanza gulf, an inshore site with some vegetation. The habitat of this group is characterized by extensive rocky (granite) boulders, general lack of aquatic vegetation and clear water with a Secchi-depth of 2 m or more (Kissenda is the exception with some vegetation and turbid water). The second genetic cluster contains all individuals from vegetation-rich sites along the eastern shore of the Mwanza Gulf, a shoreline swamp as well as Lake Burigi, Lake Rwakajunju and Lake Saka. These sampling sites have a medium to large amount of aquatic (usually emergent) vegetation, low-water clarity (Secchi-depth of less than 2 m; Supplementary Table S3) and either soft bottoms or considerable sediment cover on the rocks. The two genetic clusters of *A. alluaudi* can therefore be considered two different ecotypes. Henceforth, we refer to them as the reef ecotype and the vegetation ecotype.

To further test for phenotypic and ecological differentiation and potentially behavioral reproductive isolation between these ecotypes, and hence to test if speciation has occurred, we subsequently focus on the two populations of different ecotype that are geographically the closest to each other: The population at Makobe Island representing the reef ecotype, and the population at Sweya, representing the vegetation ecotype. These two populations show significant genomic differentiation ($F_{ST} = 0.0637$, $p < .001$).

Phenotypic divergence is pronounced between the ecotypes of *A. alluaudi*

We found significant differences between the Makobe and Sweya populations in fin length (pelvic, pectoral, caudal, dorsal and anal fin all longer in the vegetation ecotype; Figure 4; Supplementary Table S7), as well as significant differences in LJW and SnW (Supplementary Table S7), with Sweya individuals having longer fins, wider snouts and wider lower jaws than individuals from Makobe. These differences, especially in the fins, are more pronounced in males and hardly present in females (Supplementary Figures S7 and S8), and there is a pronounced sexual dimorphism (Supplementary Figure S9, Supplementary Table S7). This sexual dimorphism is stronger in *A. alluaudi* at Makobe Island than in *A. alluaudi* at Sweya (Supplementary Table S10). The PCA based on all morphological traits shows strong divergence between the two populations and ecotypes (Figure 4). PC1 is mainly explained by POW and SnW, while PC2 is mainly explained by fin length and eye size (EyD and EyL; Supplementary Table S8).

We also found a significant difference in number of anal fin spines (chi-squared = 61.83, df = 2, $p < .001$). All individuals from Sweya ($N = 16$) have either five ($N = 10$) or six ($N = 6$) anal fin spines. Five spines represent the typical anal fin spine count in this species (Greenwood, 1959). Most individuals

from Makobe, in contrast, have only four spines (N = 50), few had five (N = 7), and none had six spines (Supplementary Table S9, Supplementary Figure S5).

In our common-garden raised fish, the significant differences between the two wild populations were recovered in all traits that we could measure (anal fin length, caudal fin length and dorsal fin length; Supplementary Figure S6), indicating a heritable basis for these trait differences.

Trophic ecology is not markedly differentiated

The main prey item in both populations was snails (>50%, Supplementary Figure S10), followed by crushed mussels (~18%). In Sweya, the remaining content could not be identified, whereas for Makobe Island, a small amount of prey items consisted of small fish (7.1%), shrimps (6.8%), as well as some unidentifiable items. Generally, the prey composition of both population samples did not differ significantly (Schoener percent resource overlap index = 91.3%).

No behavioral assortative mating

The ten successful mate choice trials (four replicates with Sweya females and six with Makobe females) resulted in 123 fertilized eggs (Supplementary Table S11), which were used for parentage analyses. Although each female had the simultaneous choice between two Sweya and two Makobe males, every clutch was sired by a single male only. Two of the Sweya females mated with a Makobe male, and two mated with a Sweya male. Three of the Makobe females mated with a Sweya male, and three mated with a Makobe male (Figure 5). The generalized linear model shows no significant effect of either male ecotype, weight of the male or experimental compartment of the male, indicating random mating and no indications of any ecotype or population-based assortative female mate choice. Females on average were carrying a similar number of eggs independent of whether they mated with a male from their own or from the other ecotype and population (p = .739, Supplementary Table S11, Figure 5).

Discussion

We studied the genomic, morphological and ecological differentiation between populations of *A. alluaudi* occupying contrasting habitats. We wanted to investigate the factors that explain absence of radiation in this lineage despite its long presence in the East African Great Lakes. We found strong genetic differentiation and reciprocal monophyly among the rock and vegetation ecotypes. Surprisingly this was robust even across large geographical distances, namely including smaller lakes in the Lake Victoria and Edward catchments. Overall, ecological/habitat differences had a larger effect on genetic divergence than the geographic distance between the populations. We also detected significant phenotypic differences between two populations of the ecotypes, for instance in fin length and jaw morphology. Most of these differences between the ecotypes were retained in laboratory-bred F1 that were raised under common garden conditions. We did not detect significant differences in diet between ecotype populations. Finally, individuals of both ecotypes mated randomly in behavioral mating experiments under laboratory conditions.

Genomic divergence between habitat-specific ecotypes is stronger than isolation by geographic distance

Astatoreochromis alluaudi can be found all over Lake Victoria, the smaller lakes and swamps in its catchment and in the Lake Edward catchment, and it populates ecologically very distinct habitats (Slootweg, 1989), possibly indicating generalist habits with high dispersal rates and few dispersal limitations. High-dispersal rates can cause gene flow between populations and therefore might curtail local adaptation as well as neutral genetic divergence between populations (e.g., Arendt, 2015; Claramunt et al., 2012; Gavrilets & Vose, 2005). However, our results do not support this hypothesis. Several different populations in Lake Victoria are genetically clearly distinct from one another, and populations from the two habitat types even form reciprocally monophyletic clades. Surprisingly, the vegetation ecotype clade of southern Lake Victoria is more

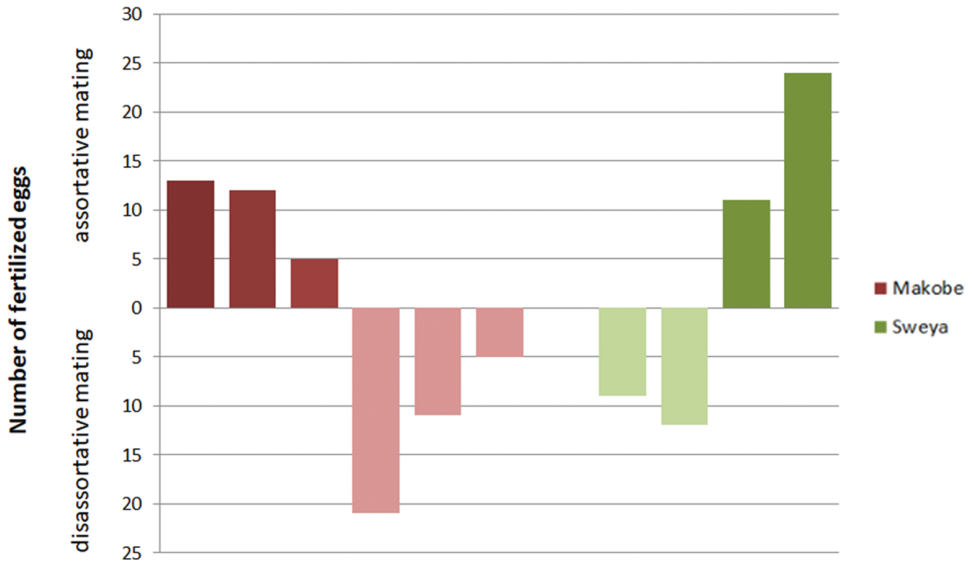

Figure 5. Number of fertilized eggs per trial. Each clutch was sired by a single male. The length of bars indicates the number of fertilized eggs: above zero are eggs resulting from matching ecotypes, below zero are eggs from matings between the two ecotypes.

closely related to individuals from vegetated habitats in geographically distant lakes (Lakes Burigi, Rwakajunju, Saka, the latter of which is in the Lake Edward catchment) than to the rocky reef ecotype clade from places nearby in southern Lake Victoria. Within Lake Victoria, we detect further subdivision within the rocky reef clade, where the populations of offshore islands form clades that are distinct from those of more nearshore islands and mainland reefs. Taken together, this indicates that the strongest genetic divergence within *A. alluaudi* occurs between populations occupying distinct habitats resulting in a strong pattern of IBE, whereas further genetic structure within the reef ecotype is probably attributed to geographic isolation, explaining the comparatively weak pattern of IBD. This pattern and the fact that the genomic divergence between the population at Makobe Island and the one in Sweya are in a similar range as between closely related species in similarly distant populations (F_{ST} = 0.097 between *Pundamilia pundamilia* from Makobe Island and *P. pundamilia*-like from Python, F_{ST} = 0.062 between *Pundamilia nyererei* from Makobe and *P. nyererei*-like from Python [Meier et al., 2017b]) indicate some barriers to gene flow between the different habitats.

Heritable phenotypic divergence between ecotypes

Species with a generalist strategy tend to undergo muted if any evolutionary phenotypic divergence between different habitats (Martin & Wainwright, 2013; Rueffler et al., 2006). As a potential habitat generalist (Binning & Chapman, 2008, Witte, 1981), *A. alluaudi* would not have to divergently adapt to the different habitats and would not be expected to diverge morphologically. Morphological divergence may be an important factor in the speciation process (Kocher, 2004) but especially so in the process of establishing sympatry between closely related species (Seehausen, 2015).

We found significant morphological differences between the two ecotypes of *A. alluaudi* in multiple traits that may play a role either in ecological adaptation (Bouton et al., 1999) or evolve under sexual selection (Baerends & Baerends, 1950). Sexual selection can differ in strength between habitats for instance due to differential predation regimes and differences in other ecological variables (Cole & Endler, 2015; Endler, 1988). This might indicate that the phenotypic differences between the ecotypes might have evolved in response to divergent ecological and sexual selection between the habitats. The fact that these differences were retained in the fish bred and raised in a common-garden environment further suggests that they have a heritable component.

Lack of assortative mating prevents speciation

The ability for sister species to establish sympatry and persist and perhaps diverge further through ecological character displacement is key for the buildup of species diversity in an adaptive radiation (Seehausen, 2015). In most animal systems, prezygotic mating barriers evolve as the first cause of reproductive isolation that may allow for persistence upon secondary contact, and eventually co-existence in sympatry (Coyne & Orr, 2004; Seehausen, 2006). One such mechanism is behavioral assortative mating, which has been suggested as being very important in rapid speciation of cichlid fish (Dominey, 1987; Knight & Turner, 2004; Maan et al., 2004; Seehausen, 2000; Selz et al., 2014b).

In our mate choice experiment with the two ecotypes, we did not detect any indication of ecotype assortative mating, despite heritable phenotypic, and genetic differences between the ecotypes. Similar experiments with member species of the Lake Victoria radiation revealed highly assortative mating (>90%) between individuals of closely related sister species that coexist in sympatry (Selz et al., 2014a, b, 2016). While our small sample size does not allow us to rule out very weak mating preferences, a signal of assortative mating similar to that in *Pundamilia* species should have shown. This indicates that the phenotypic differences between the ecotypes do not impact mating decisions, perhaps because the divergent traits are not coupled to mate choice cues. Such a lack of female preference for own ecotype despite divergent phenotypic adaptations between the ecotypes may indeed explain the lack of speciation or its completion toward persistence in sympatry in *A. alluaudi,* a constraint that has been described in several other lineages (Maan & Seehausen, 2011 and references therein).

Constraints to speciation in cichlids

Our study provides evidence for the existence of different ecotypes within the species *A. alluaudi*. These ecotypes are genetically and phenotypically distinct and occur in ecologically distinct habitats with no strong physical dispersal barriers between them. The most striking phenotypic differences were found in fin length and HW. All fins are significantly longer in individuals from Sweya than in individuals from Makobe. This pattern is retained in fish bred and raised in a common garden environment, indicating that it is heritable. It is heavily driven by the males, while these differences are less pronounced in females, reflecting the sexual dimorphism in fin length that is more pronounce in the vegetation ecotype. Male cichlids often use the display of their fins in intrasexual competition and for courtship behavior toward females (Baerends & Baerends, 1950). Especially important for courtship is the anal fin, which often displays conspicuous background coloration and contains the egg spots, which are considered an important trait for fertilization and have been suggested to play into cichlid speciation (Goldschmidt, 1991; Theis et al., 2012). Divergent sexual selection on other male nuptial traits is an important factor in haplochromine cichlid speciation (Knight & Turner, 2004; Seehausen, 2015; Seehausen et al., 2008). Significant differences in anal fin length between the males may indicate divergent sexual selection between the ecotypes (Collins et al., 2009; Debelle et al., 2014), although we were unable to find evidence for sexual selection in our female mate choice trials. The difference in spine numbers is note worthy, even though its functional importance remains unclear. Anal fin spine number is a highly conserved trait and is used to differentiate *A. alluaudi* (with 4–7 spines) from *A. straeleni* (3–4 spines) and most other haplochromine cichlids (three spines; Banyankimbona et al., 2013).

Despite pronounced genomic and phenotypic differences, *A. alluaudi* ecotypes do not show any indications of assortative mating in experimental mate choice trials. In line with this, we found evidence for considerable genomic introgression between the wild populations of the different ecotypes, mainly at sites between the range of the vegetation type and the offshore islands occupied by the reef type. Therefore, the differences between the ecotypes that accumulated in allo-parapatry do not seem to be linked to traits causing strong reproductive isolation. This is similar to the pattern detected in another cichlid lineage that occurs in Lake Tanganyika and its inlet streams which has not speciated either (Theis et al., 2014), thus pointing to the importance

of divergent sexual selection in the evolution and maintenance of the haplochromine cichlid radiations (Allender et al., 2003; Seehausen, 2015; Seehausen et al., 2008). In most cases where genetically close sister species co-occur in sympatry, there is evidence for divergent sexual selection, often triggered by male nuptial coloration sometimes in association with environmental conditions (Maan & Seehausen, 2011; Seehausen et al., 2008; Selz et al., 2014b).

Our study and previous studies (Conith et al., 2020; Dingle et al., 2008; Li et al., 2019; Lucek et al., 2014) thus provide evidence that the buildup of genomic and potentially adaptive and heritable phenotypic differences can occur rather frequently in populations living in distinct ecological habitats. However, corresponding mechanisms of reproductive isolation that would allow the transition from local adaptation and ecotype formation to speciation and persistence in sympatry do not necessarily follow. Intrinsic postzygotic reproductive isolation may evolve between allopatric populations when the populations diverge genetically over time (e.g., Ludlow & Magurran, 2006). We did not find any evidence for postzygotic isolation in *A. alluaudi*, as mating within and between ecotypes resulted in similar numbers of offspring. Prezygotic isolation, however, may arise when immigrants from an ecologically different habitat have a reduced viability (e.g., Matute et al., 2009) or reduced mating success (Snowberg & Benkman, 2009; Tobler et al., 2009). Whereas the former was not addressed in this study, we could not find evidence for the latter.

Conclusion

We discovered that *A. alluaudi*, an old occupant of the Lake Victoria basin that has never speciated, is composed of two genetically and phenotypically distinct ecotypes with wide geographical distributions. Additionally, there is genetic differentiation between populations from different rocky reefs. The genetic divergence between allopatric or allo-parapatric ecotypes is comparable in extent to that found in sympatric sister species of the radiating lineage. The differentiation is tightly linked to environmental condition rather than geographical distance among populations. The morphological differences are seen in traits that are often linked to either ecological adaptation or sexual selection. As these phenotypic differences are retained under laboratory conditions, it seems likely that they are heritable.

Despite significant phenotypic and genetic divergence, females did not show any preference for males of their own ecotype in our mate choice trials. This indicates that the identification of genomic and phenotypic differences between para- or allo-patric populations, even though of importance for management consideration, does not per se allow inferences about speciation without additional evidence for reproductive isolation in sympatry.

We conclude, that the lack of coupling between phenotypic and genetic differences on the one hand and behavioral reproductive isolation on the other hand may be a major constraint to speciation and adaptive radiation.

Supplementary material

Supplementary material is available online at *Evolution* (https://academic.oup.com/evolut/qpad001)

Data availability

RADseq reads are available at the NCBI Sequence Read Archive (SAMN23036539-SAMN32036607). Phenotypic data are available in the Figshare Data Repository DOI: 10.6084/m9.figshare.21075574. Ecological data are provided in Supplementary Material. Behavioral data are provided in the manuscript.

Ethical statement

This research was done under research permits no. 2013-251-ER-2014-177 (F.N.M.) and 2013-251-NA-2014-177 (O.S.) from the Tanzania Commission for Science and Technology (COSTECH). Behavioral experiments were done under research permits no. BE18/15 and BE65/18 from the Cantonal Veterinary Office of Bern.

Author contributions

C.M. designed and performed the mate choice experiments, dissected, and analyzed the stomachs, performed the parentage analyses, generated the geometric morphometric data, analyzed the data, and wrote the manuscript together with F.N.M. F.N.M. carried out fieldwork, participated in the design of the study, bred the common-garden fish, generated the genomic data, helped analyzing the data, wrote the manuscript together with C.M. D.F. generated the linear morphometric data. O.S. designed and coordinated the study, made the sampling design, and helped drafting the manuscript.

Conflict of interest: We have no competing interests.

Acknowledgments

We are thankful to the Tanzanian Fisheries Research Institute (TAFIRI) for hosting and supporting our fieldwork and to COSTECH for granting a research permit. We thank Jacco Van Rijssel, Salome Mwaiko, Mhoja Kayeba, Mohamed Haluna, Shane Wright, Oliver Selz, Joana Meier and Jonathan Makoye for their help in the field. We want to thank Andreas Taverna and Erwin Schaefer (animal care), Salome Mwaiko, Corinne Schmid, and Susanne Tellenbach (help in the lab), and Beat Kienholz (handiwork for the experimental set-up). Additionally, we want to thank the Genetic Diversity Center (GDC) of the ETH Zurich for providing bioinformatics facilities and the Center for Integrative Genomics at the University of Lausanne for the Sequencing. This research was funded by Swiss National Science Foundation grant 31003A_163338 to O.S.

References

Agrawal, A. A. (2001). Ecology - phenotypic plasticity in the interactions and evolution of species. *Science*, 294(5541), 321–326. https://doi.org/10.1126/science.1060701

Allender, C. J., Seehausen, O., Knight, M. E., Turner, G. F., & Maclean, N. (2003). Divergent selection during speciation of Lake Malawi cichlid fishes inferred from parallel radiations in nuptial coloration. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 14074–14079.

Arbogast, B. S., Drovetski, S. V., Curry, R. L., Boag, P. T., Seutin, G., Grant, P. R., Grant, B. R., & Anderson, D. J. (2006). The origin and diversification of Galapagos mockingbirds. *Evolution*, 60(2), 370–382.

Arendt, J. D. (2015). Effects of dispersal plasticity on population divergence and speciation. *Heredity*, 115(4), 306–311. https://doi.org/10.1038/hdy.2015.21

Baerends, G. P., & Baerends, R. (1950). An introduction to the study of the ethology of cichlid fishes. *Behaviour Supplement*, 1, 1–242.

Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., Selker, E. U., Cresko, W. A., & Johnson, E. A. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS One*, 3(10), e3376. https://doi.org/10.1371/journal.pone.0003376

Bakker, T. C. M., & Mundwiler, B. (1999). Pectoral fin size in a fish species with paternal care: A condition-dependent sexual trait revealing infection status. *Freshwater Biology*, 41, 543–551.

Banyankimbona, G., Vreven, E., & Snoeks, J. (2013). A revision of the genus *Astatoreochromis*(Teleostei, Cichlidae), East-Africa. *European Journal of Taxonomy*, 39, 1–21.

Barel, C. D. N., Van Oijen, M. J. P., Witte, F., & Wittemaas, E. L. M. (1977). Introduction to taxonomy and morphology of haplochromine cichlidae from Lake Victoria - manual to Greenwood's revision papers. *Netherlands Journal of Zoology*, 27, 333–389.

Binning, S. A., & Chapman, L. J. (2008). Feeding ecology and diet overlap in riverine cichlids from estern Uganda. *Verh. Internat. Verein. Limnol*, 30, 283–286.

Bischoff, R. J., Gould, J. L., & Rubenstein, D. I. (1985). Tail size and female choice in the guppy (*Poecilia reticulata*). *Behavior, Ecology and Sociobiology*, 17(3), 253–255. https://doi.org/10.1007/bf00300143

Bouton, N., Seehausen, O., & van Alphen, J. J. M. (1997). Resource partitioning among rock-dwelling haplochromines (Pisces: Cichlidae) from Lake Victoria. *Ecology of Freshwater Fish*, 6, 225–240.

Bouton, N., Witte, F., van Alphen, J. J. M., Schenk, A., & Seehausen, O. (1999). Local adaptations in populations of rock-dwelling haplochromines (Pisces: Cichlidae) from southern Lake Victoria. *Proceedings of the Royal Society B: Biological Sciences*, 266, 355–360.

Brawand, D., Wagner, C. E., Li, Y. I., Malinsky, M., Keller, I., Fan, S., Simakov, O., Ng, A. Y., Lim, Z. W., Bezault, E., Turner-Maier, J., Johnson, J., Alcazar, R., Noh, H. J., Russel, P., Aken, B., AlföldiJ., J., Amemiya, C., Azzouzi, N., … Di Palma, F. (2014). The genomic substrate for adaptive radiation in African cichlid fish. *Nature*, 513, 375–381.

Claramunt, S., Derryberry, E. P., Remsen, J. V., & Brumfield, R. T. (2012). High dispersal ability inhibits speciation in a continental radiation of passerine birds. *Proceedings of the Royal Society B: Biological Sciences*, 279, 1567–1574.

Cole, G. L., & Endler, J. A. (2015). Variable environmental effects on a multicomponent sexually selected trait. *American Naturalist*, 185(4), 452–468. https://doi.org/10.1086/680022

Collins, S. A., de Kort, S. R., Perez-Tris, J., & Telleria, J. L. (2009). Migration strategy and divergent sexual selection on bird song. *Proceedings of the Royal Society B: Biological Sciences*, 276, 585–590.

Conith, A. J., Kidd, M. R., Kocher, T. D., & Albertson, R. C. (2020). Ecomorphological divergence and habitat lability in the context of robust patterns of modularity in the cichlid feeding apparatus. *BMC Evolutionary Biology*, 20(1), 95. https://doi.org/10.1186/s12862-020-01648-x

Coyne, J. H. O., & Orr, H. A. (2004). *Speciation*. Sinauer.

Crother, B. I., & Guyer, C. (1996). Caribbean historical biogeography: Was the dispersal-vicariance debate eliminated by an extraterrestrial bolide? *Herpetologica*, 52, 440–465.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. Q., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R. (2011). The variant call format and VCF tools. *Bioinformatics*, 27, 2156–2158.

Debelle, A., Ritchie, M. G., & Snook, R. R. (2014). Evolution of divergent female mating preference in response to experimental sexual selection. *Evolution*, 68(9), 2524–2533. https://doi.org/10.1111/evo.12473

Dingle, C., Halfwerk, H., & Slabbekoorn, H. (2008). Habitat-dependent song divergence at subspecies level in the grey-breasted woodwren. *Journal of Evolutionary Biology*, 21, 1079–1089.

Dominey, W. J. (1987). Effects of sexual selection and life history on speciation: Species flocks in African cichlids and Hawaiian *Drosophila*. In Echelle AA, & Kornfield I. *Evolution of fish species flocks* (pp. 231–50). University of Maine at Orono Press.

Earl, D. A., & Vonholdt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4, 359–361.

Endler, J. A. (1988). Sexual selection and predation risk in guppies. *Nature*, 332(6165), 593–594. https://doi.org/10.1038/332593b0

Excoffier, L., Laval, G., & Schneider, S. (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics*, 1, 47–50.

Feller, A. F., Haesler, M. P., Peichel, C. L., & Seehausen, O. (2020). Genetic architecture of a key reproductive isolation trait differs between sympatric and non-sympatric sister species of Lake Victoria cichlids. *Proceedings of the Royal Society B: Biological Sciences*, 287, 20200270.

Gavrilets, S., & Vose, A. (2005). Dynamic patterns of adaptive radiation. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 18040–18045.

Genner, M. J., Seehausen, O., Cleary, D. F. R., Knight, M. E., Michel, E., & Turner, G. F. (2004). How does the taxonomic status of allopatric populations influence species richness within African cichlid fish assemblages? *Journal of Biogeography*, 31, 93–102.

Goldschmidt, T. (1991). Egg mimics in haplochromine cichlids (Pisces, Perciformes) from Lake Victoria. *Ethology*, 88, 177–190.

Gordon H, Hannon G. (2010). *Fastx-toolkit: FASTQ/A short-reads pre-processing tools*. http://hannonlab.cshl.edu/fastx_toolkit/download.html/fastx_toolkit-0.0.13.tar.bz2/. Accessed on April 09, 2019.

Grant, P. R., & Grant, B. R. (2008). *How and why species multiply: The radiation of Darwin's finches*. Princeton University Press.

Greenwood, P. H. (1965). Environmental effects on the pharyngeal mill of a cichlid fish, Astatoreochromis alluaudi, and their taxonomic implications. *Proceedings of the Linnean Society of London*, 176, 1–10.

Greenwood, P. H. (1959). The monotypic genera of cichlid fishes in Lake Victoria. *Bulletin of British Museum (Natural History) part II*, 5, 163–177.

Hoogerhoud, R. J. C. (1986). Taxonomic and ecological aspects of morphological plasticity in molluscivorous Haplochromines (Pisces, Cichlidae). *Ann. Mus. Roy. Afr. Centr. Sc. Zool*, 251, 131–134.

Hugall, A. F., & Stuart-Fox, D. (2012). Accelerated speciation in colour-polymorphic birds. *Nature*, 485(7400), 631–634. https://doi.org/10.1038/nature11050

Huysseune, A. (1995). Phenotypic plasticity in the lower pharyngeal jaw dentition of *Astatoreochromis alluaudi* (Teleostei, Cichlidae). *Archives of Oral Biology*, 40(11), 1005–1014. https://doi.org/10.1016/0003-9969(95)00074-y

Hyslop, E. J. (1980). Stomach contents analysis - a review of methods and their application. *Journal of Fish Biology*, 17(4), 411–429. https://doi.org/10.1111/j.1095-8649.1980.tb02775.x

Jablonski, D. (2008). Species selection: Theory and data. *Annual Review of Ecology, Evolution, and Systematics*, 39, 501–524.

Johnson, T. C., Scholz, C. A., Talbot, M. R., Kelts, K., Ricketts, R. D., Ngobi, G., Beuning, K., Ssemmanda, I., & McGill, J. W. (1996). Late pleistocene desiccation of Lake Victoria and rapid evolution of cichlid fishes. *Science*, 273(5278), 1091–1093. https://doi.org/10.1126/science.273.5278.1091

Knight, M. E., & Turner, G. F. (2004). Laboratory mating trials indicate incipient speciation by sexual selection among populations of the cichlid fish Pseudotropheus zebra from Lake Malawi. *Proceedings of the Royal Society B: Biological Sciences*, 271, 675–80

Kocher, T. D. (2004). Adaptive evolution and explosive speciation: The cichlid fish model. *Nature Reviews Genetics*, 5(4), 288–298. https://doi.org/10.1038/nrg1316

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, 9(4), 357–359. https://doi.org/10.1038/nmeth.1923

Li, M. R., Wang, H. Y., Ding, N., Lu, T., Huang, Y. C., Xiao, H. X., Liu, B., & Li, L. F. (2019). Rapid divergence followed by adaptation to contrasting ecological niches of two closely related columbine species *Aquilegia japonica* and *A. oxysepala*. *Genome Biology and Evolution*, 11(3), 919–930. https://doi.org/10.1093/gbe/evz038

Losos, J. B. (2009). *Lizards in an evolutionary tree: Ecology and adaptive radiation of anoles*. University of California Press.

Lucek, K., Sivasundar, A., Kristjánsson, B. K., Skúlason, S., & Seehausen, O. (2014). Quick divergence but slow convergence during ecotype formation in lake and stream stickleback pairs of variable age. *Journal of Evolutionary Biology*, 27(9), 1878–1892. https://doi.org/10.1111/jeb.12439

Ludlow, A. M., & Magurran, A. E. (2006). Gametic isolation in guppies (*Poecilia reticulata*). *Proceedings of the Royal Society B: Biological Sciences*, 273, 2477–2482.

Maan, M. E., & Seehausen, O. (2011). Ecology, sexual selection and speciation. *Ecology Letters*, 14(6), 591–602. https://doi.org/10.1111/j.1461-0248.2011.01606.x

Maan, M. E., Seehausen, O., Soderberg, L., Johnson, L., Ripmeester, E. A. P., Mrosso, H. D. J., Taylor, M. I., van Dooren, T. J. M., & van Alphen, J. J. M. (2004). Intraspecific sexual selection on a speciation trait, male coloration, in the Lake Victoria cichlid *Pundamilia nyererei*. *Proceedings of the Royal Society B: Biological Sciences*, 271, 2445–2452.

Marques, D. A., Lucek, K., Meier, J. I., Mwaiko, S., Wagner, C. E., Excoffier, L., & Seehausen, O. (2016). Genomics of rapid incipient speciation in sympatric threespine stickleback. *PLoS Genetics*, 12(2), e1005887. https://doi.org/10.1371/journal.pgen.1005887

Martin, C. H., & Wainwright, P. C. (2013). Multiple fitness peaks on the adaptive landscape drive adaptive radiation in the wild. *Science*, 339(6116), 208–211. https://doi.org/10.1126/science.1227710

Matute, D. R., Novak, C. J., & Coyne, J. A. (2009). Temperature-based extrinsic reproductive isolation in two species of drosophila. *Evolution*, 63(3), 595–612. https://doi.org/10.1111/j.1558-5646.2008.00588.x

Mbabazi, D., Ogutu-Ohwayo, R., Wandera, S. B., & Kiziito, Y. (2004). Fish species and trophic diversity of haplochromine cichlids in the Kyoga satellite lakes (Uganda). *African Journal of Ecology*, 42, 59–68.

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., & DePristo, M. A. (2010). The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, 20(9), 1297–1303. https://doi.org/10.1101/gr.107524.110

Meier, J. I., Marques, D. A., Mwaiko, S., Wagner, C. E., Excoffier, L., & Seehausen, O. (2017a). Ancient hybridization fuels rapid cichlid fish adaptive radiations. *Nature Communications*, 8, 14363. https://doi.org/10.1038/ncomms14363

Meier, J. I., Sousa, V. C., Marques, D. A., Selz, O. M., Wagner, C. E., Excoffier, L., & Seehausen, O. (2017b). Demographic modelling with whole-genome data reveals parallel origin of similar Pundamilia cichlid species after hybridization. *Molecular Ecology*, 26(1), 123–141. https://doi.org/10.1111/mec.13838

Moser, F. N., van Rijssel, J. C., Mwaiko, S., Meier, J. I., Ngatunga, B., & Seehausen, O. (2018). The onset of ecological diversification 50 years after colonization of a crater lake by haplochromine cichlid fishes. *Proceedings of the Royal Society B: Biological Sciences*, 285, 20180171.

Moser, F. (2018). Investigating ecological and genetic constraints to speciation. [Unpublished Doctoral Thesis] *philosophisch-naturwissenschaftliche Fakultät der Universität Bern*.

Nonaka, E., Svanback, R., Thibert-Plante, X., Englund, G., & Brannstrom, A. (2015). Mechanisms by which phenotypic plasticity affects adaptive divergence and ecological speciation. *American Naturalist*, 186(5), E126–E143. https://doi.org/10.1086/683231

Parsons, K. J., Robinson, B. W., & Hrbek, T. (2003). Getting into shape: An empirical comparison of traditional truss-based morphometric methods with a newer geometric method applied to New World cichlids. *Environmental Biology of Fishes*, 67, 417–431.

Pfennig, D. W., Rice, A. M., & Martin, R. A. (2006). Ecological opportunity and phenotypic plasticity interact to promote character displacement and species coexistence. *Ecology*, 87(3), 769–779. https://doi.org/10.1890/05-0787

Pfennig, D. W., Wund, M. A., Snell-Rood, E. C., Cruickshank, T., Schlichting, C. D., & Moczek, A. P. (2010). Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology and Evolution*, 25(8), 459–467. https://doi.org/10.1016/j.tree.2010.05.006

Plaut, I. (2000). Effects of fin size on swimming performance, swimming behaviour and routine activity of zebrafish Danio rerio. *Journal of Experimental Biology*, 203(Pt 4), 813–820. https://doi.org/10.1242/jeb.203.4.813

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155(2), 945–959. https://doi.org/10.1093/genetics/155.2.945

Qu, Y., Duan, M., Yan, J., Feng, G., & Liu, J., et al. (2013). Effects of lateral morphology on swimming performance in two sturgeon species. *Journal of Applied Ichthyology*, 29, 310–315.

R-Core-Team. 2015. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Rabosky, D. L. (2016). Reproductive isolation and the causes of speciation rate variation in nature. *Biological Journal of the Linnean Society*, 118, 13–25.

Rabosky, D. L., Santini, F., Eastman, J., Smith, S. A., Sidlauskas, B., Chang, J., & Alfaro, M. E. (2013). Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nature Communications*, 4, 1958. https://doi.org/10.1038/ncomms2958

Rohlf, F. J. (2015). The tps series of software. *Hystrix*, 26, 9–12.

Rolland, J., Condamine, F. L., Jiguet, F., & Morlon, H. (2014). Faster speciation and reduced extinction in the tropics contribute to the mammalian latitudinal diversity gradient. *PLoS Biology*, 12, e100.

Ronco, F., Matschiner, M., Böhne, A., Boila, A., Büscher, H. H., El Taher, A., Indermaur, A., Malinsky, M., Ricci, V., Kahmen, A., Jentoft, S., & Salzburger, W. (2020). Drivers and dynamic of a massive adaptive radiation in cichlid fishes. *Nature*, 589(7840), 76–81. https://doi.org/10.1038/s41586-020-2930-4

Rueffler, C., Van Dooren, T. J. M., Leimar, O., & Abrams, P. A. (2006). Disruptive selection and then what?. *Trends in Ecology and Evolution*, 21(5), 238–245. https://doi.org/10.1016/j.tree.2006.03.003

Sambrook, J., & Russell, D. 2001. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory Press.

Sanetra, M., Henning, F., Fukamachi, S., & Meyer, A. (2009). A microsatellite-based genetic linkage map of the cichlid fish, *Astatotilapia burtoni* (Teleostei): A comparison of genomic architectures among rapidly speciating cichlids. *Genetics*, 182(1), 387–397. https://doi.org/10.1534/genetics.108.089367

Schluter, D. 2000. *The ecology of adaptive radiation*. Oxford Series in Ecology and Evolution.

Schluter, D., & Pennell, M. W. (2017). Speciation gradients and the distribution of biodiversity. *Nature*, 546(7656), 48–55. https://doi.org/10.1038/nature22897

Schneider, R. F., & Meyer, A. (2017). How plasticity, genetic assimilation and cryptic genetic variation may contribute to adaptive radiations. *Molecular Ecology*, 26(1), 330–350. https://doi.org/10.1111/mec.13880

Schoener, T. W. (1970). Nonsynchronous spatial overlap of lizards in patchy habitats. *Ecology*, 51(3), 408–418. https://doi.org/10.2307/1935376

Seehausen, O. (2000). Explosive speciation rates and unusual species richness in haplochromine cichlid fishes: Effects of sexual selection. *Advances in Ecological Research*, 31, 237–274.

Seehausen, O. (2002). Patterns in fish radiation are compatible with Pleistocene desiccation of Lake Victoria and 14 600 year history for its cichlid species flock. *Proceedings of the Royal Society B: Biological Sciences*, 269, 491–497.

Seehausen, O. (2006). African cichlid fish: A model system in adaptive radiation research. *Proceedings of the Royal Society B: Biological Sciences*, 273, 1987–1998.

Seehausen O. 2009. Progressive levels of trait divergence along a "speciation transect" in the Lake Victoria cichlid fish *Pundamilia*. *Speciation and patterns of diversity* (pp. 155–76). Cambridge University Press.

Seehausen, O. (2015). Process and pattern in cichlid radiations - inferences for understanding unusually high rates of evolutionary diversification. *New Phytologist*, 207(2), 304–312. https://doi.org/10.1111/nph.13450

Seehausen, O., & Bouton, N. (1997). Microdistribution and fluctuations in niche overlap in a rocky shore cichlid community in Lake Victoria. *Ecology of Freshwater Fish*, 6(3), 161–173. https://doi.org/10.1111/j.1600-0633.1997.tb00159.x

Seehausen, O., Terai, Y., Magalhaes, I. S., Carleton, K. L., Mrosso, H. D. J., Miyagi, R., van der Sluijs, I., Schneider, M. V., Maan, M. E., Tachida, H., Imai, H., & Okada, N. (2008). Speciation through sensory drive in cichlid fish. *Nature*, 455(7213), 620–626. https://doi.org/10.1038/nature07285

Selz, O. M., Thommen, R., Maan, M. E., & Seehausen, O. (2014a). Behavioural isolation may facilitate homoploid hybrid speciation in cichlid fish. *Journal of Evolutionary Biology*, 24, 275–289.

Selz, O. M., Pierotti, M. E. R., Maan, M. E., Schmid, C., & Seehausen, O. (2014b). Female preference for male color is necessary and sufficient for assortative mating in 2 cichlid sister species. *Behavioral Ecology*, 25(3), 612–626. https://doi.org/10.1093/beheco/aru024

Selz, O. M., Thommen, R., Pierotti, M. E. R., Anaya-Rojas, J. M., & Seehausen, O. (2016). Differences in male coloration are predicted by divergent sexual selection between populations of a cichlid fish. *Proceedings of the Royal Society B: Biological Sciences*, 283, 20160172.

Slootweg, R. (1989). Proposed introduction of *Astatoreochromis alluaudi*, an East African mollisk crushing cichlid, as a mean of snail control. *Musee Royal de l'Afrique Central, Annales Sciences Zoologique*, 257, 61–64.

Smits, J. D., Witte, F., & VanVeen, F. G. (1996). Functional changes in the anatomy of the pharyngeal jaw apparatus of *Astatoreochromis alluaudi* (Pisces, Cichlidae), and their effects on adjacent structures. *Biological Journal of the Linnean Society*, 59, 389–409.

Snowberg, L. K., & Benkman, C. W. (2009). Mate choice based on a key ecological performance trait. *Journal of Evolutionary Biology*, 22(4), 762–769. https://doi.org/10.1111/j.1420-9101.2009.01699.x

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Taylor, M. I., Meardon, F., Turner, G., Seehausen, O., Mrosso, H. D. J., & Rico, C. (2002). Characterization of tetranucleotide microsatellite loci in a Lake Victorian, haplochromine cichlid fish: A *Pundamilia pundamilia* x *Pundamilia nyererei* hybrid. *Molecular Ecology Notes*, 2(4), 443–445. https://doi.org/10.1046/j.1471-8286.2002.00272.x

Theis, A., Ronco, F., Indermaur, A., Salzburger, W., & Egger, B. (2014). Adaptive divergence between lake and stream populations of an East African cichlid fish. *Molecular Ecology*, 23(21), 5304–5322. https://doi.org/10.1111/mec.12939

Theis, A., Salzburger, W., & Egger, B. (2012). The function of anal fin egg-spots in the cichlid fish Astatotilapia burtoni. *PLoS One*, 7(1), e29878. https://doi.org/10.1371/journal.pone.0029878

Tobler, M., Riesch, R., Tobler, C. M., Schulz-Mirbach, T., & Plath, M. (2009). Natural and sexual selection against immigrants maintains differentiation among micro-allopatric populations. *Journal of Evolutionary Biology*, 22, 2298–2304.

Turner, G. F. (2007). Adaptive radiation of cichlid fish. *Current Biology*, 17, R827827–R82R831. https://doi.org/10.1016/j.cub.2007.07.026

Turner, G. F., Seehausen, O., Knight, M. E., Allender, C. J., & Robinson, R. L. (2001). How many species of cichlid fishes are there in African lakes?. *Molecular Ecology*, 10(3), 793–806. https://doi.org/10.1046/j.1365-294x.2001.01200.x

Wagner, C. E., Harmon, L. J., & Seehausen, O. (2012). Ecological opportunity and sexual selection together predict adaptive radiation. *Nature*, 487(7407), 366–369. https://doi.org/10.1038/nature11144

Wagner, C. E., Harmon, L. J., & Seehausen, O. (2014). Cichlid species-area relationships are shaped by adaptive radiations that scale with area. *Ecology Letters*, 17(5), 583–592. https://doi.org/10.1111/ele.12260

Weir, J. T., & Schluter, D. (2007). The latitudinal gradient in recent speciation and extinction rates of birds and mammals. *Science*, 315(5818), 1574–1576. https://doi.org/10.1126/science.1135590

Witte, F., & Van Oijen, M. J. P. (1990). Taxonomy, ecology and fishery of Lake Victoria haplochromine trophic groups. *Zoologische Verhandelingen*, 262, 1–47.

Witte, F. (1981). Initial results of the ecological survey of the haplochromine cichlid species from the Mwanza Gulf of Lake Victoria (Tanzania): breeding patterns, trophic and species distribution. *Neth. J. Zool*, 31, 175–202.

Young, R. L. (2013). Linking conceptual mechanisms and transcriptomic evidence of plasticity-driven diversification. *Molecular Ecology*, 22(17), 4363–4365. https://doi.org/10.1111/mec.12467

Zheng, X. W., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, 28(24), 3326–3328. https://doi.org/10.1093/bioinformatics/bts606