

SCIENTIFIC REPORTS



OPEN

Genetic evidence for panmixia in a colony-breeding crater lake cichlid fish

T. K. Lehtonen^{1,2}, K. R. Elmer^{1,3}, M. Lappalainen³ & A. Meyer¹

Fine-scaled genetic structuring, as seen for example in many lacustrine fish, typically relates to the patterns of migration, habitat use, mating system or other ecological factors. Because the same processes can also affect the propensity of population differentiation and divergence, assessments of species from rapidly speciating clades, or with particularly interesting ecological traits, can be especially insightful. For this study, we assessed the spatial genetic relationships, including the genetic evidence for sex-biased dispersal, in a colony-breeding cichlid fish, *Amphilophus astorquii*, endemic to Crater Lake Apoyo in Nicaragua, using 11 polymorphic microsatellite loci ($n = 123$ individuals from three colonies). We found no population structure in *A. astorquii* either within colonies (no spatial genetic autocorrelation, $r \sim 0$), or at the lake-wide level (pairwise population differentiation $F_{ST} = 0-0.013$ and no clustering), and there was no sex-bias (male and female A_{IC} values bounded 0) to this lack of genetic structure. These patterns may be driven by the colony-breeding reproductive behaviour of *A. astorquii*. The results suggest that strong philopatry or spatial assortative mating are unlikely to explain the rapid speciation processes associated with the history of this species in Lake Apoyo.

Dispersal, when resulting in gene flow, is a fundamental process in evolution. In interaction with other behavioural characteristics and demography, it can exert a large influence on genetic population structure^{1,2}. Molecular methods have revealed very fine scaled genetic structuring among many animal populations, especially those of lacustrine fish³⁻⁷. These patterns often result from low levels of migration, a lack of significant larval dispersal, and a tendency for philopatry by the adults. Interestingly, high levels of such philopatric behaviour have been found across a wide range of taxa, including insects, fish, amphibians, birds and mammals⁸⁻¹⁰.

Patterns of population structure may also relate to habitat use and feeding preferences, with trophic and habitat specialists often exhibiting more restricted gene flow over any habitat discontinuities than more opportunistic species^{4,11-13}. However, such ecological barriers are not necessarily required to induce philopatric behaviour^{14,15}. For example, in *Variabilichromis moorii*, a monogamous Lake Tanganyikan cichlid fish, mitochondrial DNA sequences indicated that dispersal is very infrequent and at similar levels along continuous stretches of rocky shore and across localities separated by sandy habitat¹⁴. Dispersal patterns affecting the population genetic structure can also be sex specific. For example, in superb fairy-wrens, *Malurus cyaneus*, that exhibit fine-scale genetic structure, any male dispersal from their natal territory occurs only over very short distances, whereas female fairy-wrens show also relatively long distance dispersal^{2,16}. In cichlid fish of Lake Malawi, in turn, gene flow occurs primarily by dispersal of sub-adult males that search for breeding territories, with females tending to stay in their natal area^{15,17}. As a result, kin structuring of the population may be evident only within the philopatric sex, typically males in birds and females in mammals and fish⁸. Such sex-biased dispersal may be related to avoidance of the risk of inbreeding, competition with kin, sex biased co-operation with kin, or sex specific territorial behaviour^{8,18-21}. More generally, mating system characteristics, such as the patterns of territoriality and mating competition, can markedly contribute to the genetic structure^{15,22-25}.

Factors that affect dispersal and smaller scale genetic structure can also influence the likelihood of speciation²⁶⁻³¹. This is especially true for philopatry: for example the outstandingly species rich radiations of cichlid fish in the African Great Lakes have been associated with philopatric tendencies, as well as genetic differentiation

¹Zoology and Evolutionary Biology, Department of Biology, University of Konstanz, 78457, Konstanz, Germany.

²School of Biological Sciences, Monash University, Victoria, 3800, Australia. ³Institute of Biodiversity, Animal Health & Comparative Medicine, College of Medical, Veterinary & Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK. T. K. Lehtonen and K. R. Elmer contributed equally to this work. Correspondence and requests for materials should be addressed to A.M. (email: axel.meyer@uni-konstanz.de)

over short distances within the lakes^{3,4,14}. Researchers have also suggested that groups with a greater population genetic structure should be more diverse than clades without fine-scale structure, as reduction in gene flow may be linked to isolation¹². On the other hand, lacustrine fish are also known to be particularly prone to speciate in sympatry³², with pronounced differences in speciation rates between even closely related clades^{12,33}. Therefore, to investigate the importance of different speciation mechanisms, studies on the present genetic structure in clades that are exceptionally fast (or slow) to speciate can be particularly informative^{12,34,35}.

In this study, we assessed the population structure at different spatial scales, as well as the genetic evidence for sex-biased dispersal, in a cichlid fish, *Amphilophus astorquii*, which is endemic to Crater Lake Apoyo in Nicaragua. *Amphilophus astorquii* was chosen for the study for multiple reasons. First, it is a species from a particularly rapidly speciating cichlid clade that inhabits mostly Nicaraguan freshwaters, with six endemic species of the group living in Lake Apoyo alone^{33,36,37}. Indeed, similarly to its congeners in Nicaraguan crater lakes, *A. astorquii* seems to have evolved through sympatric speciation^{38,39}. Second, the species is notable for breeding in dense breeding aggregations, while also showing high levels of territorial competition with its conspecific, and occasionally heterospecific, neighbours⁴⁰. Third, it has a more benthic life style than *Amphilophus zaliosus*, a closely related and sympatric pelagic species³⁸. In this regard, pelagic cichlids are often thought to have less population structure than those using more benthic habitats^{13,41,42}. Finally, at the time of the study, *A. astorquii* was locally the most abundant breeding cichlid species^{40,43}. Such colony-like breeding aggregations allowed sampling at different levels of physical distance, in order to make interferences about migration and gene flow within the lake. Specifically we tested: (a) whether there is genetic differentiation between breeding colonies, and if so, whether such patterns are sex-biased, either of which would suggest site fidelity in the scale of the whole lake; and (b) whether there is a spatial pattern of relatedness within colonies, which would suggest fine-scale site fidelity and/or kin-grouping influencing spatial distributions.

Materials and Methods

The sampling was conducted in Lake Apoyo. The crater lake formed approximately 23 000 years ago⁴⁴ and is the largest of its kind in Nicaragua, with diameter of ~5 km, shoreline of ~20 km and maximum depth of 175 m⁴⁵. Our model species, *A. astorquii*, is endemic to the lake, where it arose by sympatric speciation from a generalist ancestor^{38,46}. It is the most distinctly colony-breeding species in the Midas cichlid complex, in which males and females are thought to pair monogamously around the same time they claim a territory⁴⁷ and then provide parental care until their fry are approximately six weeks old. The fish may survive to breed during more than one breeding season (with the same or a new social partner).

Aggregations of *A. astorquii* breeding pairs, from here on referred to as ‘colonies’ (defined as an area of a high concentration of breeding pairs with no, or very few, breeding individuals in the intercepting area between colonies) within Lake Apoyo were associated with beds of *Chara* algae. *Chara* does not occur everywhere in the lake, contributing to the patchiness of different habitat types (i.e. sandy and predominantly rocky areas with and without *Chara*). The colonies were the densest at depths of 4–8 metres, with each pair maintaining a territory of approximately 0.5 m diameter, this also being the minimum distance between adjoining territories (Fig. 1).

Sampling fishes. Several locations around the lake were pre-surveyed within the limitations of time constraints, divers’ access to the lake from the shore and reasonable dive/swim times from these access points. Five to six colonies were identified, and logistic limitations allowed sampling of three representative colonies (Fig. 1) that were both accessible and large enough for our sampling procedure (see below for details). The samples were collected in December 2007, during the peak of the breeding season, which in this species usually lasts from November to January^{40,48}. All colonies were associated with thick *Chara* beds (Fig. 1) in areas of gentle to moderately steep depth slopes at depths of 3.5–8 metres. Prior to sampling, SCUBA divers set a transect line, roughly parallel to the shore line, through each colony. Social pairs (males and females) were then collected from 3 m intervals along the full length of each transect, using hand nets (Fig. 1), with harpoons as a back-up. When approached, the pair withdrew to the centre of their territory within *Chara* growth (Fig. 1). The net was then rapidly pushed against the substratum, capturing the pair. In total, 123 adult individuals were collected and genotyped from the three colonies (Fig. 1): Colony 1 n = 63 (30 pairs, 2 females, 1 male); Colony 2 n = 23 (10 pairs, 2 females, 1 male); Colony 3 n = 37 (16 pairs, 3 females, 2 males).

Genotyping. Genomic DNA was isolated from ethanol-preserved tissue using standard proteinase K digestion followed by high salt extraction methods, resuspended in water and stored at –20 °C. Eleven microsatellite loci were amplified using standard PCR conditions: Abur151, Abur82, Abur28, Abur45⁴⁹, M1M, M2, M7, M12⁵⁰, Unh012, Unh013⁵¹, and TmoM7⁵². Ten percent of individuals were repeated as technical replicates. For each locus, one primer was labelled with a NED, FAM or HEX fluorescent dye (Applied Biosystems) for subsequent visualization. Loci were genotyped in an ABI 3130xl (Applied Biosystems) and sized according to Rox standard (Applied Biosystems).

Genotyping was quality controlled for errors and null alleles in Micro-checker⁵³. Analyses of all samples in a single population suggested a low presence of null alleles (excess homozygosity) at Abur 28 (0.06 Brookfield frequency in homozygotes), Abur45 (0.17), and Unh012 (0.14)⁵⁴. However this signal was not found when each colony was tested separately (i.e. in no case did each colony have null alleles inferred by Micro-checker), suggesting it is not a pervasive bias in the data and so all loci were retained. To verify the inferred pattern, analyses that might be particularly sensitive to homozygosity were also conducted using a reduced number of loci (see Results). Data are available in Table S3.

Population genetic analyses. Summary statistics of diversity and population structure were calculated in GenoDive v2.0b27⁵⁵ and statistical significance assessed with 9999 permutations. Infinite allele model was

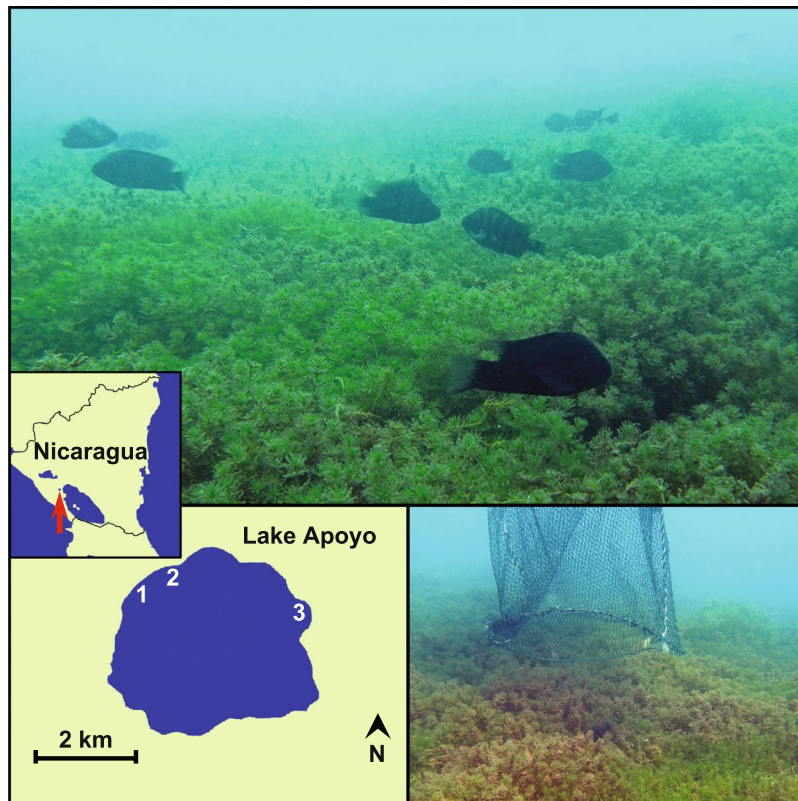


Figure 1. Colony 1 amidst a thick bed of *Chara* algae (top section, photo taken by TKL on 18/December/2007), outline of Lake Apoyo with locations of the sampled *Amphilophus astorquii* colonies (bottom left, drawn by TKL in Adobe Photoshop Elements 4.0, <http://www.adobe.com/products/photoshop-elements.html>), a sampling net that was quickly placed over a pair seeking shelter within *Chara* (bottom right, photo taken by TKL on 13/December/2007).

assumed for calculating the analysis of molecular variance (AMOVA). F_{IS} statistics were calculated in GenePop on the Web v4.2 (Markov chain parameters: dememorization 1000, batches 100, iterations per batch 1000).

Spatial genetic autocorrelation was assessed over the three 'populations' in Genalex v 6.501⁵⁶. Genetic and geographic distance matrices were calculated for each of the three colonies using 11 loci and significance assessed with 999 permutations. Distance classes were set at 3 (for the 3 m intervals sampled along transects from 0 to 102 m) and the number of distance classes set at 30.

Genetic variation was visualised with an individuals-based Principal Coordinates Analysis (PCoA) of genotypic distances (co-variance-standardized method) in GenALEX. A genetic clustering analysis was conducted in Structure v 2.4.3⁵⁷, with model setting for correlated allele frequencies and admixture among populations without population information (no pop flag), run for 600,000 MCMC generations after 100,000 generations burning. Six replicates were run for $K = 1$ through $K = 4$, and log-likelihood results plotted in Structure Harvester⁵⁸.

Sex-biased dispersal was tested in Genalex. As that test tolerates no missing genotypes, we replaced any missing data with the two most common alleles at that locus (0 to 19 individuals across loci) and removed one individual and one locus (TmoM7), with a higher proportion of missing data, in colony 3.

MARENA Nicaragua issued the necessary permits for the study (permit numbers 026/-11007/DGAP and DGRNB-IC-006-2007) and it was performed in accordance with the laws of Nicaragua.

Data availability. Data are made available in Table S3.

Results

Summary statistics. Observed heterozygosity was 0.510 ± 0.073 and heterozygosity within populations 0.576 ± 0.073 (Table S1). Based on the full microsatellite dataset of 11 loci, analysing the lake as a population resulted in deviation from HWE ($\chi^2 = \text{infinity}$, $Df = 62$, Prob = High sign). Each colony also deviated significantly from HWE when assessed separately, though there was no strong pattern across loci and colonies, with the exception of high F_{IS} in Unh012 for all colonies (Table S2).

Population differentiation. An F -statistic-based AMOVA analysis suggested there was little population genetic differentiation among colonies, with inter-population variation contributing less than 1% of the total variation (Table 1). This inference is robust to locus-specific deviations from HWE, as a reduced analysis excluding loci with significant deviations (excluding Abur28, Abur45, Unh012) yielded the same AMOVA results (97.3%

Source of Variation	%var		F-value	±SD	P
Within Individual	90.8%	F_{IT}	0.092	0.034	—
Among Individual Within populations	8.4%	F_{IS}	0.085	0.034	0
Among Population	0.7%	F_{ST}	0.007	0.005	0.011

Table 1. Analysis of molecular variance based on infinite allele model, across individuals and colonies with F-statistics corresponding to Weir and Cockerham⁷⁰.

	Pop1	Pop2	Pop3
Pop1	—	0.003	0.013*
Pop2	100.007	—	−0.005
Pop3	136.214*	83.901	—

Table 2. Population differentiation from F_{ST} values (above diagonal) and log likelihood G-statistic values (below diagonal) for all pairwise comparisons. Significant differences after Bonferroni correction (0.05/3) are noted with *. However, excluding the three loci with deviations from HWE results in no pairwise population comparison being significant.

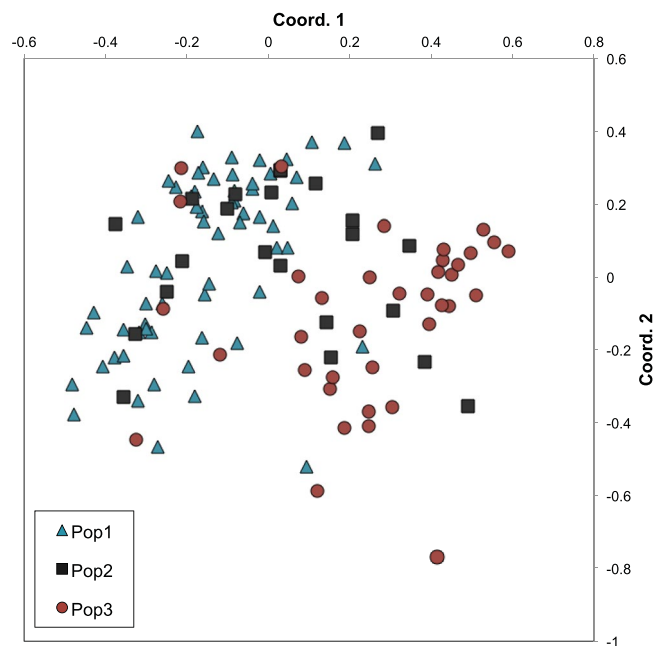


Figure 2. Principal coordinates analysis of individual variation visualised on the first and second axis, including all *A. astorquii* from colonies (populations) 1, 2, and 3.

variation among individuals, 2.1% among individuals within populations, 0.6% among populations; $F_{ST} = 0.006$, $p = 0.034$). The PCoA plot (axis 1 vs axis 2) shows some weak separation along coordinate 1 between colony 1 and 2 vs. colony 3, but considerable overlap of individuals from different colonies (Fig. 2). A Structure analysis to infer genetic clusters suggests $K = 1$ as the most likely genetic grouping, with any other grouping ($K > 1$) being poorly supported, with lower likelihood and higher standard deviation (Fig. S1). Visualisation of the admixture proportions for all individuals at more than one group showed no clustering, with all individuals being equally admixed to two or three groups ($Q \sim 50\%$ or 33% for each individual; not shown).

Population genetic differentiation from a pairwise analysis between colonies was also low, with F_{ST} between populations ranging from 0 to 0.013, in agreement with the findings from the AMOVA analysis. With permutation there was significant differentiation between Colony 1 and Colony 3 (Table 2), though the absolute values were low. If loci deviating significantly from HWE (Abur28, Abur45, Unh012) were excluded from the analysis, the population differentiation between colonies 1 and 3 was not significant based on the log-likelihood G (G -statistic = 79.298, $p = 0.18$) but remained significant based on F_{ST} ($F_{ST} = 0.012$; $p = 0.009$). Overall this suggests very low population differentiation between colonies.

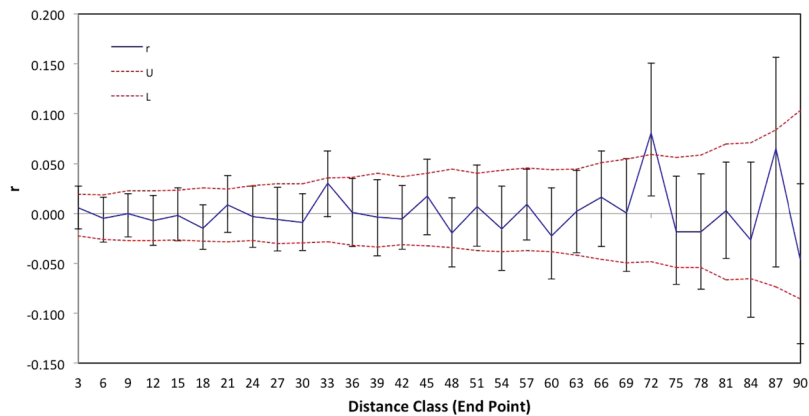


Figure 3. Correlogram of the linear spatial genetic relationship (r) among individuals within colonies, assessed at 3 m distance classes. Upper and lower error bars bound the 95% confidence interval for r as determined by bootstrap resampling. Upper and lower confidence limits (dashed lines) bound the 95% confidence on the null hypothesis of no spatial genetic structure.

Spatial genetic structure. No spatial genetic structure was detected within colonies. Inter-individual differentiation at all distance classes was non-significant except at 72 m intervals (bias corrected $r = 0.081$, $p < 0.003$) but as this is only one distance class at high linear distance we do not consider it biologically significant. Importantly, in all other distance classes, r falls within the upper and lower bounds and the error bars on r always include 0 (Fig. 3).

The test for heterogeneity, which would indicate a deviation of spatial genetic correlation relationships across all colonies, is non-significant (combined correlogram $\omega = 88.58$, $p = 0.015$ when $p < 0.01$ is significant). When distance classes are reduced to 15, to only include the maximum class at which there are data from all 3 colonies (as the linear length of colonies differs), there is still no evidence for spatial genetic autocorrelation ($\omega = 37.89$, $p = 0.16$).

Sex-biased dispersal. We found no evidence for sex biased dispersal in *A. astorquii*, reflected as no significant difference between the mean AIC (Assignment Index correction) values for males vs. females in assignment tests. Both male $AIC = 0.190 \pm 0.275$ and female $AIC = -0.172 \pm 0.234$, (mean \pm SE) bounded zero, and no difference between the two sexes was found (U-test of difference between males and females: two tailed probably for $|Z| = 0.211$, non-significant) (Fig. S2).

Discussion

The scope of this study was to assess within and between colony population genetic structure in *A. astorquii*, a colony-breeding cichlid fish living in Nicaragua's largest crater lake, Lake Apoyo. The results show no spatial genetic structure within the three breeding *A. astorquii* colonies, contrary to what might have been predicted under site fidelity or kin-clustering. Similarly, there was little or no population differentiation among the colonies; the lake is effectively a panmictic population. Furthermore, there was no sex-bias to this lack of genetic structure. The lack of genetic differences among colonies suggests that there is no colony fidelity in *A. astorquii*. Either adult individuals commonly settle into different breeding colonies for different breeding seasons or, after becoming independent, juveniles disperse away from their natal colony. In other words, the seasonal aggregations of breeders may temporally alternate with more widely ranging life-history phases. In this respect, the results are similar to many generalist and pelagic cichlid species^{4,12,13}, including *A. zalius* from Lake Apoyo³⁸ and *Amphilophus xiloaensis* from nearby crater lake Xiloá⁵⁹ that were not found to show any significant population structure across different sites within the lakes. However, our results are in contrast with previous studies on African cichlids that have shown that benthic (and other specialised) species tend to have moderate to high levels of spatial genetic structuring, including high fidelity to breeding grounds and an inhibition to crossing habitats^{3,4,12,60–62}.

Because more pronounced geographic structure has been found in fish at scales much smaller than the size of Lake Apoyo^{3,4,61}, the size of the lake alone cannot explain the observed lack of population structure in these cichlids. One potential explanation for this difference could be fluctuations in the existence and distribution of *Chara* algae beds where all *A. astorquii* breeding grounds were located at the time of this study (Fig. 1). In particular, the occurrence of *Chara* in this lake varies over time⁶³ (personal observations 2007–2016), and hence the location of breeding aggregations associated with *Chara* may not remain stable enough for philopatry to evolve or for any population structuring to build up detectable genetic signal. This hypothesis is also in line with our finding that there was no sex-bias to the lack of structure between or within the colonies, while earlier studies on African cichlids have suggested that dispersal in cichlids can be either male^{15,17,64} or female⁶⁵ biased.

Amphilophus astorquii belongs to the extremely rapidly speciating lineage of cichlid fish, and it has speciated sympatrically within a few hundred generations after the formation of Lake Apoyo (approximately 23000 years ago⁴⁴) within the lake^{39,46}. Our results are therefore relevant also in the context of inferring the most likely modes of rapid speciation taking place within the lake. The comparison between colonies from different parts of the lake suggests that there is no biologically significant level of population structure within the species. This makes the occurrence of parapatric speciation via strong 'homing' unlikely³⁰. Indeed, together with the previous studies that found no significant population structure within other Nicaraguan crater lake cichlids^{38,56}, the current study

provides evidence against the existence of barriers to gene flow within the lake. The lack of such barriers is in accordance with the proposition of sympatric speciation being important in the origin of these species^{36,38,39}. Other modes of speciation, e.g. through disruptive natural selection, have been proposed to explain elevated speciation rates in taxa showing less genetic population-structure^{66–68} and are also likely to play an important role in the (sympatric) origin of cichlid species in Nicaraguan crater lakes, such as Lake Apoyo^{38,39,69}.

To conclude, we found no population structure in *A. astorquii* either at the colony or lake-wide level. The results are important in suggesting that strong philopatry or sex biased dispersal are unlikely to contribute markedly to the rapid speciation process the species has undergone within Lake Apoyo, providing further support for sympatric ecological speciation as the driver of the extremely rapid diversification of these cichlids in Nicaraguan crater lakes in Nicaragua.

References

- Cockburn, A. Cooperative breeding in oscine passerines: Does sociality inhibit speciation? *Proceedings of the Royal Society B* **270**, 2207–2214 (2003).
- Double, M. C., Peakall, R., Beck, N. R. & Cockburn, A. Dispersal, philopatry, and infidelity: dissecting local genetic structure in superb fairy-wrens (*Malurus cyaneus*). *Evolution* **59**, 625–635 (2005).
- Rico, C. & Turner, G. F. Extreme microallopatric divergence in a cichlid species from Lake Malawi. *Molecular Ecology* **11**, 1585–1590 (2002).
- Pereyra, R., Taylor, M. I., Turner, G. F. & Rico, C. Variation in habitat preference and population structure among three species of the Lake Malawi cichlid genus *Protomelas*. *Molecular Ecology* **13**, 2691–2697 (2004).
- Barluenga, M. & Meyer, A. Old fish in a young lake: stone loach (Pisces: *Barbatula barbatula*) populations in Lake Constance are genetically isolated by distance. *Molecular Ecology* **14**, 1229–1239 (2005).
- Adams, C. E. *et al.* Does breeding site fidelity drive phenotypic and genetic sub-structuring of a population of Arctic charr? *Evolutionary Ecology* **20**, 11–26 (2006).
- Bergek, S. & Björklund, M. Cryptic barriers to dispersal within a lake allow genetic differentiation of Eurasian perch. *Evolution* **61**, 2035–2041 (2007).
- Hatchwell, B. J. Cryptic kin selection: kin structure in vertebrate populations and opportunities for kin-directed cooperation. *Ethology* **116**, 203–216 (2010).
- Refsnider, J. M. & Janzen, F. J. Putting eggs in one basket: Ecological and evolutionary hypotheses for variation in oviposition-site choice. *Annual Review of Ecology, Evolution, and Systematics* **41**, 39–57 (2010).
- Stearns, F. W., Tilmon, K. J. & Wood, T. K. Felsenstein's "one-allele model" of speciation: The role of philopatry in the initial stages of host plant mediated reproductive isolation in *Enchenopa binotata*. *Current Zoology* **59**, 658–666 (2013).
- Rüber, L., Verheyen, E. & Meyer, A. Replicated evolution of trophic specializations in an endemic cichlid fish lineage from Lake Tanganyika. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 10230–10235 (1999).
- Wagner, C. E. & McCune, A. R. Contrasting patterns of spatial genetic structure in sympatric rock-dwelling cichlid fishes. *Evolution* **63**, 1312–1326 (2009).
- Kobl Müller, S., Odhiambo, E. A., Sinyinza, D., Sturmbauer, C. & Sefc, K. M. Big fish, little divergence: phylogeography of Lake Tanganyika's giant cichlid, *Boulengerochromis microlepis*. *Hydrobiologia* **748**, 29–38 (2015).
- Duftner, N. *et al.* Distinct population structure in a phenotypically homogeneous rock-dwelling cichlid fish from Lake Tanganyika. *Molecular Ecology* **15**, 2381–2395 (2006).
- Anseeuw, D. *et al.* Subtle population structure and male-biased dispersal in two *Copadichromis* species (Teleostei, Cichlidae) from Lake Malawi, East Africa. *Hydrobiologia* **615**, 69–79 (2008).
- Mulder, R. A. Natal and breeding dispersal in a co-operative, extra-group mating bird. *Journal of Avian Biology* **26**, 234–240 (1995).
- Knight, M. E., Turner, G. F., van Oppen, M. J. H., Rico, C. & Hewitt, G. M. Evidence for male-biased dispersal in Lake Malawi cichlids from microsatellites. *Molecular Ecology* **8**, 1521–1527 (1999).
- Queller, D. C. Does population viscosity promote kin selection? *Trends in Ecology and Evolution* **7**, 322–324 (1992).
- Taylor, P. D. Inclusive fitness in a heterogeneous environment. *Proceedings of the Royal Society B* **249**, 299–302 (1992).
- Keller, L. F. & Waller, D. M. Inbreeding effects in wild populations. *Trends in Ecology and Evolution* **17**, 230–241 (2002).
- West, S. A., Pen, I. & Griffin, A. S. Conflict and cooperation – cooperation and competition between relatives. *Science* **296**, 72–75 (2002).
- van Oppen, M. J. H. *et al.* Assortative mating among rock-dwelling cichlid fishes supports high estimates of species richness from Lake Malawi. *Molecular Ecology* **7**, 991–1001 (1998).
- Perrin, N. & Mazalov, V. Local competition, inbreeding and the evolution of sex-biased dispersal. *The American Naturalist* **155**, 116–127 (2000).
- Avise, J. C. *et al.* Genetic mating systems and reproductive natural histories of fishes: Lessons for ecology and evolution. *Annual Review of Genetics* **36**, 19–45 (2002).
- Sefc, K. M., Baric, S., Salzburger, W. & Sturmbauer, C. Species-specific population structure in rock-specialized sympatric cichlid species in Lake Tanganyika, East Africa. *Journal of Molecular Evolution* **64**, 33–49 (2007).
- Barraclough, T. G., Harvey, P. H. & Nee, S. Sexual selection and taxonomic diversity in passerine birds. *Proceedings of the Royal Society B* **259**, 211–215 (1995).
- Mitra, S., Landel, H. & Jones, S. P. Species richness covaries with mating system in birds. *Auk* **113**, 544–551 (1996).
- Owens, I. P. F., Bennett, P. M. & Harvey, P. H. Species richness among birds: body size, life history, sexual selection or ecology? *Proceedings of the Royal Society B* **266**, 933–939 (1999).
- Arnqvist, G., Edvardsson, M., Friberg, U. & Nilsson, T. Sexual conflict promotes speciation in insects. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 10460–10464 (2000).
- Gavrilets, S. Models of speciation: what have we learned in 40 years? *Evolution* **57**, 2197–2215 (2003).
- Matschiner, M., Hanel, R. & Salzburger, W. 2010. Phylogeography and speciation processes in marine fishes and fishes from large freshwater lakes. In *Phylogeography. Concepts, intraspecific patterns and speciation processes* (ed. Rutgers, D. S.) 1–29. (Nova Science Publishers, New York, 2010).
- Seehausen, O. & Wagner, C. E. Speciation in freshwater fishes. *Annual Review of Ecology, Evolution, and Systematics* **45**, 621–651 (2014).
- Elmer, K. R., Kusche, H., Lehtonen, T. K. & Meyer, A. Local variation and parallel evolution: morphological and genetic diversity across a species complex of neotropical crater lake cichlid fishes. *Philosophical Transactions of the Royal Society B* **365**, 1763–1782 (2010).
- Maeda, K. *et al.* Population structure of two closely related pelagic cichlids in Lake Victoria, *Haplochromis pyrrhocephalus* and *H. laparogramma*. *Gene* **441**, 67–73 (2009).
- Takeda, M. *et al.* Genetic structure of pelagic and littoral cichlid fishes from Lake Victoria. *PLoS ONE* **8**, e74088 (2013).
- Wilson, A. B., Noack-Kunmann, K. & Meyer, A. Incipient speciation in sympatric Nicaraguan crater lake cichlid fishes: sexual selection versus ecological diversification. *Proceedings of the Royal Society B* **267**, 2133–2141 (2000).

37. Barluenga, M. & Meyer, A. Phylogeography, colonization and population history of the Midas cichlid species complex (*Amphilophus* spp.) in the Nicaraguan crater lakes. *BMC Evolutionary Biology* **10**, 326 (2010).
38. Barluenga, M., Stölting, K. N., Salzburger, W., Muschick, M. & Meyer, A. Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature* **439**, 719–723 (2006).
39. Kautt, A. F., Machado-Schiaffino, G. & Meyer, A. Multispecies outcomes of sympatric speciation after admixture with the source population in two radiations of Nicaraguan Crater Lake cichlids. *PLoS Genetics* **12**, e1006157 (2016).
40. Lehtonen, T. K., McCrary, J. K. & Meyer, A. Territorial aggression can be sensitive to the status of heterospecific intruders. *Behavioural Processes* **84**, 598–601 (2010).
41. Shaw, P. W., Turner, G. F., Idid, M. R., Robinson, R. L. & Carvalho, G. R. Genetic population structure indicates sympatric speciation of Lake Malawi pelagic cichlids. *Proceedings of the Royal Society B* **267**, 2273–2280 (2000).
42. Taylor, M. I. & Verheyen, E. Microsatellite data reveals weak population substructuring in *Copadichromis* sp. 'virginalis kajose', a demersal cichlid from Lake Malawi, Africa. *Journal of Fish Biology* **59**, 593–604 (2001).
43. Lehtonen, T. K., McCrary, J. K. & Meyer, A. Introduced predator elicits deficient brood defence behaviour in a crater lake fish. *PLoS ONE* **7**, e30064 (2012).
44. Sussman, D. Apoyo caldera, Nicaragua: A major Quaternary silicic eruptive center. *Journal of Volcanology and Geothermal Research* **24**, 249–282 (1985).
45. INETER. Laguna de Apoyo. Retrieved 5 Apr, 2009 from, <http://www.ineter.gov.ni/Direcciones/Recursos%20Hidricos/HIDROGRAFIA%20WEB/Lagunas/Apoyo.htm> (2009).
46. Elmer, K. R. *et al.* Parallel evolution of Nicaraguan crater lake cichlid fishes by non-parallel routes. *Nature Communications* **5**, 6168 (2014).
47. Barlow, G. W. The benefits of being gold: behavioral consequences of polychromatism in the midas cichlid. *Cichlasoma citrinellum*. *Environmental Biology of Fishes* **8**, 235–247 (1983).
48. Stauffer, J. R. Jr., McCrary, J. K. & Black, K. E. Three new species of cichlid fishes (Teleostei: Cichlidae) from Lake Apoyo, Nicaragua. *Proceedings of the Biological Society of Washington* **121**, 117–129 (2008).
49. Sanetra, M., Henning, E., Fukamachi, S. & Meyer, A. A microsatellite-based genetic linkage map of the cichlid fish, *Astatotilapia burtoni* (Teleostei): a comparison of genomic architectures among rapidly speciating cichlids. *Genetics* **182**, 387–397 (2009).
50. Noack, K., Wilson, A. B. & Meyer, A. Broad taxonomic applicability of microsatellites developed for the highly polymorphic neotropical cichlid. *Amphilophus citrinellum* *Animal Genetics* **31**, 151–152 (2000).
51. McKaye, K. R. *et al.* Behavioral, morphological and genetic evidence of divergence of the Midas cichlid species complex in two Nicaraguan crater lakes. *Cuadernos de Investigación de la UCA* **12**, 19–47 (2002).
52. Zardoya, R. *et al.* Evolutionary conservation of microsatellite flanking regions and their use in resolving the phylogeny of cichlid fishes (Pisces: Perciformes). *Proceedings of the Royal Society B* **263**, 1589–1598 (1996).
53. van Oosterhout, C., Hutchison, W. F., Wills, D. P. M. & Shipley, P. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535–538 (2004).
54. Brookfield, J. F. Y. A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology* **5**, 453–455 (1996).
55. Meirmans, P. G. & Van Tienderen, P. H. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* **4**, 792–794 (2004).
56. Peakall, R. & Smouse, P. E. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288–295 (2006).
57. Falush, D., Stephens, M. & Pritchard, J. K. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164**, 1567–1587 (2003).
58. Earl, D. A. & vonHoldt, B. M. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**, 359–361 (2012).
59. Elmer, K. R., Lehtonen, T. K. & Meyer, A. Color assortative mating contributes to sympatric divergence of neotropical cichlid fish. *Evolution* **63**, 2750–2757 (2009).
60. Verheyen, E., Rüber, L., Snoeks, J. & Meyer, A. Mitochondrial phylogeography of rock-dwelling cichlid fishes reveals evolutionary influence of historical lake level fluctuations of Lake Tanganyika, Africa. *Philosophical Transactions of the Royal Society B* **351**, 797–805 (1996).
61. van Oppen, M. J. H. *et al.* Unusually fine-scale genetic structuring found in rapidly speciating Malawi cichlid fishes. *Proceedings of the Royal Society B* **264**, 1803–1812 (1997).
62. Arnegard, M. E. *et al.* Population structure and colour variation of the cichlid fish *Labeotropheus fuelleborni* Ahl along a recently formed archipelago of rocky habitat patches in southern Lake Malawi. *Proceedings of the Royal Society B* **266**, 119–130 (1999).
63. McCrary, J. K., Murphy, B. R., Stauffer, J. R. Jr. & Hendrix, S. S. Tilapia (Teleostei: Cichlidae) status in Nicaraguan natural waters. *Environmental Biology of Fishes* **78**, 107–114 (2007).
64. Dierkes, P., Heg, D., Taborsky, M., Skubic, E. & Achmann, R. Genetic relatedness in groups is sex-specific and declines with age of helpers in a cooperatively breeding cichlid. *Ecology Letters* **8**, 968–975 (2005).
65. Taylor, M. I., Morley, J. I., Rico, C. & Balshine, S. Evidence of genetic monogamy and female-biased dispersal in the parental mouthbrooding cichlid *Eretmodus cyanostictus* from Lake Tanganyika. *Molecular Ecology* **12**, 3173–3177 (2003).
66. Turner, G. F. Speciation mechanisms in Lake Malawi cichlids: a critical review. *Advances in Limnology* **44**, 139–160 (1994).
67. Turner, G. F. & Burrows, M. T. A model of sympatric speciation by sexual selection. *Proceedings of the Royal Society B* **260**, 287–292 (1995).
68. Danley, P. D. & Kocher, T. D. Speciation in rapidly diverging systems: lessons from Lake Malawi. *Molecular Ecology* **10**, 1075–1086 (2001).
69. Machado-Schiaffino, G. *et al.* Incipient speciation driven by hypertrophied lips in Midas cichlids fish? *Molecular Ecology* **26**, 2348–2362 (2017).
70. Weir, B. S. & Cockerham, C. C. Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370 (1984).

Acknowledgements

We thank M. Geiger and L. López Pérez for assistance in the field, J. McCrary for logistical support in the field, and B. Rueter, E. Dillenius, E. Hespeler, and H. Recknagel for assistance in the lab. This research was funded by Alexander von Humboldt Foundation (KRE and TKL), NSERC (KRE), and DFG (AM).

Author Contributions

K.R.E. and T.K.L. conceived and designed the study, T.K.L. gathered the field data, K.R.E. performed the laboratory work and genotyping, M.L. and K.R.E. conducted the analyses, T.K.L. and K.R.E. wrote the first draft of the manuscript with A.M. providing edits and advice. All authors played a role in preparing the final version of the manuscript and reviewed it.

Additional Information

Supplementary information accompanies this paper at <https://doi.org/10.1038/s41598-018-19266-5>.

Competing Interests: The authors declare that they have no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2018