

Please cite the Published Version

Freudiger, Annika, Josi, Dario, Thünken, Timo, Herder, Fabian, Flury, Jana M, Marques, David A, Taborsky, Michael and Frommen, Joachim G (2021) Ecological variation drives morphological differentiation in a highly social vertebrate. *Functional Ecology*, 35 (10). pp. 2266-2281. ISSN 0269-8463

DOI: <https://doi.org/10.1111/1365-2435.13857>

Publisher: Wiley

Version: Published Version

Downloaded from: <https://e-space.mmu.ac.uk/630297/>

Usage rights:  [Creative Commons: Attribution-Noncommercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/)

Additional Information: This is an Open Access article which appeared in *Functional Ecology*, published by Wiley

Enquiries:

If you have questions about this document, contact openresearch@mmu.ac.uk. Please include the URL of the record in e-space. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from <https://www.mmu.ac.uk/library/using-the-library/policies-and-guidelines>)

RESEARCH ARTICLE

Functional Ecology



Ecological variation drives morphological differentiation in a highly social vertebrate

Annika Freudiger^{1,2} | Dario Josi^{1,2} | Timo Thünken^{1,3} | Fabian Herder⁴ |
Jana M. Flury⁴ | David A. Marques^{5,6} | Michael Taborsky¹ | Joachim G. Frommen^{1,2}

¹Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, Hinterkappelen, Switzerland; ²Conservation Ecology, Evolution and Behaviour Research Group, Ecology and Environment Research Centre, Department for Natural Sciences, Manchester Metropolitan University, Manchester, UK; ³Institute of Evolutionary Biology and Ecology, University of Bonn, Bonn, Germany; ⁴Sektion Ichthyologie, Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany; ⁵Division of Aquatic Ecology and Evolution, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland and ⁶Department of Fish Ecology and Evolution, Centre for Ecology and Evolution, and Biochemistry, Swiss Federal Institute of Aquatic Science and Technology (EAWAG), Kastanienbaum, Switzerland

Correspondence

Annika Freudiger

Email: annika.freudiger@iee.unibe.ch

Funding information

Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: 310030B_138660, 310030_185021, 31003A_156152, 31003A_166470 and 31003A_176174

Handling Editor: Thomas Houslay

Abstract

1. Animals may respond to ecological heterogeneity by genetic differentiation or phenotypic plasticity. Responses of organisms to their ecology can include adaptation at various levels of organization, including morphology, behaviour and social structure. Adaptations at one level might constrain or enhance adaptations on other levels, which highlights the importance of understanding their interactions.
2. In highly social animals, understanding the influence of their ecological niche on the evolution and maintenance of complex social organization poses an intriguing challenge. Predation risk and habitat characteristics determine social structure in the cooperatively breeding cichlid *Neolamprologus pulcher*. Here we examine how varying combinations of these ecological factors across eight distinct populations influence morphological differentiation.
3. We investigated the relationship between body shape and ecological parameters for 137 wild-caught individuals from eight distinct populations. Furthermore, we examined the genetic structure and differentiation among these populations using microsatellites. Finally, to disentangle heritable from plastic responses, we raised two successive generations from six populations in the laboratory under common garden conditions and screened 188 individuals for morphological differentiation.
4. We found that body shape of *N. pulcher* strongly correlates with the measured ecological parameters. Low predation risk, low habitat structure and small shelter size favoured shallow bodies, whereas at the opposite end of these environmental gradients deep body shapes prevail. These consistent morphological differences persisted over two laboratory-reared generations, revealing a heritable basis. In contrast to the significant effect of local ecology on morphological differentiation between populations, both geographical and genetic distance had

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. *Functional Ecology* published by John Wiley & Sons Ltd on behalf of British Ecological Society

little explanatory power, suggesting that morphological differentiation between populations is not a simple by-product of genetic isolation. Remarkably, the largest difference in body depth emerged between the two populations located closest to each other, but differing strongly in their ecological niche.

5. These results highlight that morphological variation is a key component of local adaptation in neighbouring populations of a highly social species. Such morphological differentiation has the potential to influence individual cooperative behaviour, which will eventually feed back on group structure and mediate the evolution and maintenance of complex social systems.

KEYWORDS

cichlid fish, cooperative breeding, eco-morphology, local adaptation, *Neolamprologus pulcher*

1 | INTRODUCTION

Adaptations to ecological conditions may include morphological and physiological attributes, behavioural traits, social structure or a combination thereof (DeWitt et al., 1999; Heynen et al., 2017; Koenig, 1981; Willmer et al., 2005). These adaptations may reinforce or compensate each other (DeWitt et al., 1999; Heynen et al., 2017; Marshall & Wund, 2017; Mushagalusa et al., 2019). For instance, under high predation pressure, freshwater snails (*Physa gyrina*) evolved narrow shell apertures rendering them less vulnerable to shell-entry predation. Along with the resulting reduced vulnerability, individuals with narrow shell apertures were also found to be bolder and to show more antipredator behaviour (DeWitt et al., 1999). In social animals, variable ecological factors may directly affect social interactions by changing the costs and benefits of group living (Arnold & Owens, 1999; Curry, 1989; Koenig et al., 2011; Marshall et al., 2016). In addition, ecological variation may trigger adaptations in other phenotypic traits, which may, in turn, feed back on social organization (Cannizzo et al., 2019; Zuk et al., 1998). For example, parasite infections lead to changes in female red junglefowl's *Gallus gallus* comb size, which is accompanied with a shift in hierarchy so that infected females lose their dominance and consequently attain a lower social status (Zuk et al., 1998). Therefore, it seems appropriate to study ecological effects on different attributes of organisms to understand their mutual influence on the striking diversity of social structures.

Adaptations to the local ecology may lead to consistent phenotypic differences between populations. Two distinct processes are commonly assumed to be responsible for such divergence. First, physical or geographical restrictions to gene flow may cause genetic differences between populations through genetic drift, which may result in phenotypic differentiation as a by-product (Bolnick & Otto, 2013; Slatkin, 1993; Wright, 1943). Second, genetic differentiation may result from divergent natural selection between populations, leading to phenotypic adaptations to the local ecology (Nosil et al., 2005; Schluter, 2000; Sexton et al., 2014; Slatkin, 1993; Wang & Summers, 2010). However, in fluctuating habitats, the

fitness effects of different phenotypes may considerably diverge within or between generations (Bradshaw, 1965; Miner et al., 2005; Ruehl & DeWitt, 2005; West-Eberhard, 2003). Fluctuating selection pressures should hence diminish genetic differentiation between populations by maintaining heritable phenotypic variation within populations (DeWitt & Scheiner, 2004; Nosil et al., 2018). The ability to plastically respond to a particular environment by expressing appropriate phenotypes (Bradshaw, 1965; DeWitt & Scheiner, 2004; McCollum & Van Buskirk, 1996) can happen either within the life span of an individual (Young et al., 2003) or across generations (Agrawal et al., 1999; DeWitt, 1998). In any case, adjustments of this sort typically involve individual behavioural responses that may strongly affect social organization (Cantor et al., 2020; Groenewoud et al., 2016).

Morphological attributes of organisms seem less plastic than, for instance, behaviour. They might hence be assumed to vary primarily due to genetic differentiation (Falconer, 1981). Still, it has been well established that phenotypic plasticity may be similarly important for local morphological adaptation (Krueger & Dodson, 1981; Rohner & Moczek, 2020; West-Eberhard, 1989). Fishes are one of the best studied groups showing remarkable differentiation in morphology as response to various ecological factors, which can be linked to heritable or plastic responses (e.g. Eklöv & Jonsson, 2007; Imre et al., 2002; Olsson & Eklöv, 2005; Riesch et al., 2018; Ruehl et al., 2011). Among the ecological factors driving morphological differentiation are predation risk and habitat complexity, which may induce changes in body shape (Brönmark & Miner, 1992; Frommen et al., 2011; Ghalambor et al., 2004; Stauffer & van Snik Gray, 2004; Taylor & McPhail, 1985, 1986). For example, under high risk, fishes often show a deep body rendering various potential benefits, including reduced handling success by gape-size limited predators (Brönmark & Miner, 1992; Nilsson & Brönmark, 2000; Nilsson et al., 1995; Weber et al., 2012), or a higher burst swimming performance and enhanced turning rate (Domenici et al., 2008; Langerhans & Reznick, 2010; Webb, 1982, 1984). Improved swimming performance is especially advantageous in highly structured environments (Langerhans, 2009; Schrank et al., 1999; Walker, 1997; Webb, 1984;

Webb et al., 1996; Webb & Weihs, 1986). On the contrary, in species relying on access to shelters to hide and breed, body depth may be constrained by the size of available shelters (Gashagaza et al., 1995; Takahashi et al., 2009).

Fishes are renowned for their impressive diversity in social organization, ranging from solitary life or assembling in loose shoals to long-lasting, complex societies (Godin, 1997; Hara & Pitcher, 1986; Taborsky, 2016). This social diversity is linked to variation in biotic (e.g. predation pressure) and abiotic factors (e.g. habitat type; for review, see Taborsky & Wong, 2017). For example, fishes living in long-lasting social groups may adjust group size (Bergmüller et al., 2005; Taborsky, 1985; Zöttl et al., 2013) or group structure (Groenewoud et al., 2016; Tanaka et al., 2016) to adapt to the prevailing conditions. Such adjustments in behaviour and social organization may coincide with divergence in other traits varying with local conditions, including morphology. The covariance between behavioural and morphological attributes across different populations exposed to deviating ecological conditions is hitherto not well understood.

The cooperatively breeding cichlid *Neolamprologus pulcher* offers great opportunities to study the interplay between morphology and sociality in animals. *N. pulcher* is endemic to Lake Tanganyika where it breeds colonially in ecologically diverse benthic habitats along the sublittoral zone (Balshine et al., 2001; Groenewoud et al., 2016; Heg et al., 2005). Colonies are stable over several years (Heg et al., 2005; Stiver et al., 2004) and individual dispersal distances rarely exceed 20 m (Stiver et al., 2004, 2007). Groups consist of a breeding pair, which is on average assisted by five to six helpers of different age cohorts (Balshine et al., 2001; Groenewoud et al., 2016; Stiver et al., 2004; Taborsky, 2016). Within groups, helpers engage in direct brood care, territory maintenance and defence (Balshine-Earn et al., 1998; Bergmüller et al., 2005; Taborsky, 2016). Territories usually contain either clefts in rocks, burrows under stones or empty gastropod shells providing shelter and serving as breeding chambers. The territory is defended against hetero- and conspecific intruders, which is mainly done by large group members (Bruintjes & Taborsky, 2011; Groenewoud et al., 2016; Jungwirth et al., 2015). Variation in ecological settings (including predation risk, habitat complexity and substrate quality) has pronounced impact on the behaviour and group structure of *N. pulcher* in populations living in different habitats (Groenewoud et al., 2016).

Here we ask whether multidimensional differences in the ecological niches of distinct populations affect morphological attributes, which may relate to the established variation in social structure of *N. pulcher*. We used three different approaches. First, we quantified the morphological differentiation of wild-caught individuals from eight populations varying in predation risk and structural complexity. We compared morphological and ecological data to assess whether local adaptation might explain potential morphological differentiation. Second, we tested for neutral genetic differentiation (i.e. variance in 13 microsatellites distributed across the genome that are unlikely to have fitness effects) between these populations to examine whether potential morphological differentiation might be a by-product of restricted gene flow and genetic drift. If morphological differentiation

arose by restrictions in gene flow, we would expect to find a correlation between morphological and neutral genetic differentiation between populations. Third, to disentangle plastic responses from heritable variation, we collected specimens from the different populations and compared wild-caught, F1 and F2 fish raised under common garden conditions in the laboratory.

2 | MATERIALS AND METHODS

2.1 | Study species

2.1.1 | Study populations

We selected eight ecologically distinct populations of *N. pulcher* at the southern tip of Lake Tanganyika, near Mpulungu, Zambia, according to marked differences in rock cover and predator abundance (for details see Section 2.2; Table 1; Figure 1b). Throughout the manuscript, we refer to these populations by the numbers 1–8, which correspond to the scores of the ecological niche axis in an ascending manner (for details, see Section 2.2; Figure 2). The eight populations were 140 m to 21 km apart from each other (Table S1). Two of these populations are geographically clearly separated from the others, whereas the other six cluster in two groups of three populations each (Figure 1a).

2.1.2 | Samples

For genetic analysis, we collected tissue samples of 34 breeder-sized individuals in each population from September to November 2014 and 2015. Individuals were caught haphazardly using fence nets over the complete colony, and a small part (approx. 1 mm by 2 mm) of their dorsal or anal fin was removed and preserved in 98% ethanol. Afterwards, they were released again.

For geometric morphometric analyses, we collected 151 individuals of these eight ecologically distinct populations from September to November 2012 and 2013 using fence nets. We haphazardly selected fish from all parts of the study populations. We measured the standard length (SL) of each specimen and determined its sex by visual inspection of the genital papilla. Males only have one opening (urinary pore), whereas females have two (urinary pore and oviduct; Popma & Masser, 1999). The fish were euthanized with an overdose of KOI MED® Sleep and preserved in buffered 4% formalin. Determining the sex was not possible for one small individual, which was therefore excluded from further analyses.

2.1.3 | Common garden experiment

In December 2012 we brought wild-caught individuals from six of the eight study populations (populations 1, 3, 4, 5, 7 and 8) to the laboratory at the Ethologische Station Hasli (Bern, Switzerland). We

TABLE 1 Summary of sample sizes used for morphological analyses, with additional information on the numbers of females (F) and males (M) and the respective range of body sizes (SL), and ecological parameters measured for each study population. Given are arithmetic means \pm standard deviations. Populations are sorted by geographical location from West to East, population numbers indicate their position on the ecological niche axis

Population	Wild-caught <i>n</i> and SL (mm)	F1 <i>n</i> and SL (mm)	F2 <i>n</i> and SL (mm)	Predation risk	Shelter size (mm)	Sand cover (%)	Ecological niche
4	5F (26–30) 10M (25–37)	9F (50–73) 7M (55–66)	5F (28–42) 6M (29–40)	3.58 ± 1.86	17.63 ± 2.71	38.06	−0.590
3	28F (31–56) 25M (31–65)	9F (50–61) 8M (55–70)	9F (29–43) 9M (31–42)	1.31 ± 0.38	19.14 ± 2.69	36.60	−0.779
8	4F (38–54) 9M (34–56)	9F (44–62) 8M (53–70)	4F (30–39) 0M (NA)	5.56 ± 1.13	28.60 ± 2.29	6.17	2.254
5	5F (33–49) 1M (39)	9F (48–69) 9M (54–71)	5F (30–40) 4M (40–42)	0.75 ± 0.50	22.16 ± 3.20	10.83	0.444
2	8F (31–50) 10M (33–52)	NA	NA	0.17 ± 0.30	15.56 ± 1.81	44.17	−1.691
1	11F (29–45) 6M (29–45)	7F (43–64) 8M (54–78)	13F (30–42) 6M (29–41)	0.00 ± 0.00	11.84 ± 1.85	45.76	−2.212
6	0F (NA) 11M (57–70)	NA	NA	4.65 ± 2.30	24.03 ± 5.07	22.00	0.957
7	3F (27–49) 1M (37)	8F (55–71) 10M (53–80)	14F (28–41) 10M (32–43)	7.75 ± 3.21	23.21 ± 3.08	17.19	1.618

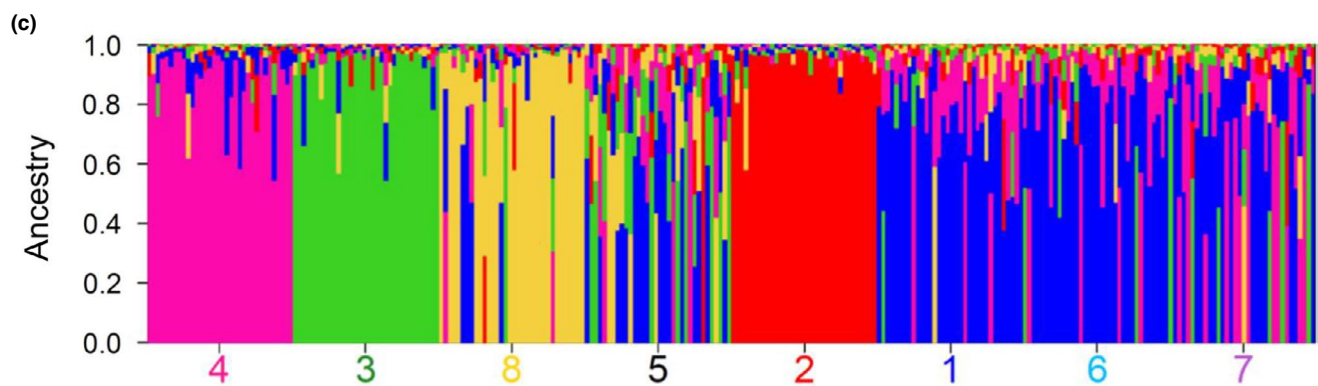
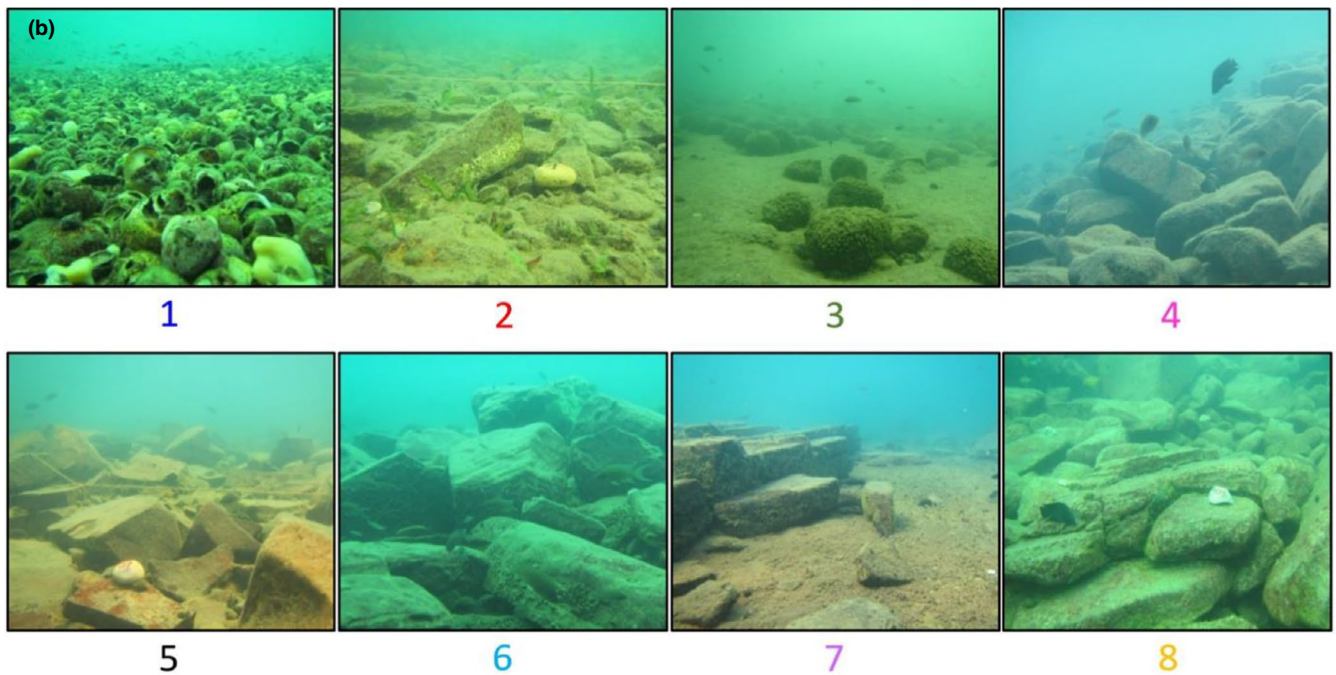
used these fish to breed first F1 and subsequently F2 individuals, which we raised under common garden conditions (see ‘Appendix S1’ for detailed description of housing conditions). We sampled 119 F1 individuals originating from 75 families (specimens per family: range = 1–4, median = 2) and 102 F2 individuals originating from 49 families (specimens per family: range = 1–8, median = 2). We noted SL and sex of each individual and euthanized them with an overdose of KOI MED® Sleep before fixing them in buffered 4% formalin. Sexing was not possible for three F2 individuals, because they had not reached sexual maturity, and were hence excluded from further analyses.

2.2 | Ecological measures

From September to November in the years 2012–2015, we measured ecological parameters of the eight populations by SCUBA diving. We focussed on predation risk, shelter size and structural complexity, as these parameters had been shown to influence the morphology of Lake Tanganyika cichlids and other fishes (e.g. Domenici et al., 2008; Langerhans & Reznick, 2010; Nilsson & Brönmark, 2000; Takahashi et al., 2009). The methods have been described in detail by Groenewoud et al. (2016) and Josi et al. (2018). In brief, four transects of 1 m \times 10 m

were established by laying sisal fibre ropes on the lake bottom, starting from the centre of each population. Predation risk was assessed by counting the number of predatory fishes (*Lepidolamprologus elongatus*, *Lepidolamprologus attenuatus* and *Lamprologus lemairei*) along the four transects. Each transect was surveyed between 6 and 10 times per population on different days, to account for variation in fish activity. The mean number of large (>10 cm) *L. elongatus* and *L. attenuatus* per population served as a proxy for predation risk, as these were the most common species, posing a risk to *N. pulcher* of all sizes. We used sand cover as a measure of structural complexity of the habitats, with sandy stretches being flat and showing little variation in the third dimension, while rocky patches exhibit much more variation in the third dimension. The percentage of sand cover was determined in every square meter of the four transects, and we calculated the means per site. Within each colony, we randomly selected shelters inhabited by *N. pulcher* ($n_{\text{tot}} = 127$, range: $n = 10$ –21 per population) and measured the size of the shelter entrances. Following the methods described in Tanaka et al. (2016), a transparent plastic sheet was rolled to a cylinder and inserted into the shelter entrance. There, it was slowly unrolled, until the diameter of the cylinder equalled the diameter of the shelter entrance, and measured with a ruler (Table 1). We constructed a model of the ecological niches by combining these three factors into principal components (PCs). Given the strong correlation of the three

FIGURE 1 Geography rather than ecology drives population structure and genetic differentiation in *Neolamprologus pulcher*. Ancestry proportions of wild-caught specimens to population clusters determined with the software STRUCTURE in relation to the geographical location of the populations. (a) The study sites were located at the southern tip of Lake Tanganyika. (b) Photos exemplifying differences in shelter size and habitat structure of the eight study populations in Lake Tanganyika. (c) The genetic data fit to a five-cluster model, which had the highest likelihood. Populations are sorted by geographical location from West to East, population numbers indicate their position on the ecological niche axis. Each vertical coloured bar corresponds to one specimen and each colour represents a distinct genetic cluster. Populations 2, 3, 4 and 8 are made up mainly by one genetic cluster each; population 5 is a mixture of various genetic clusters. Populations 1, 6 and 7 are indistinguishable from each other



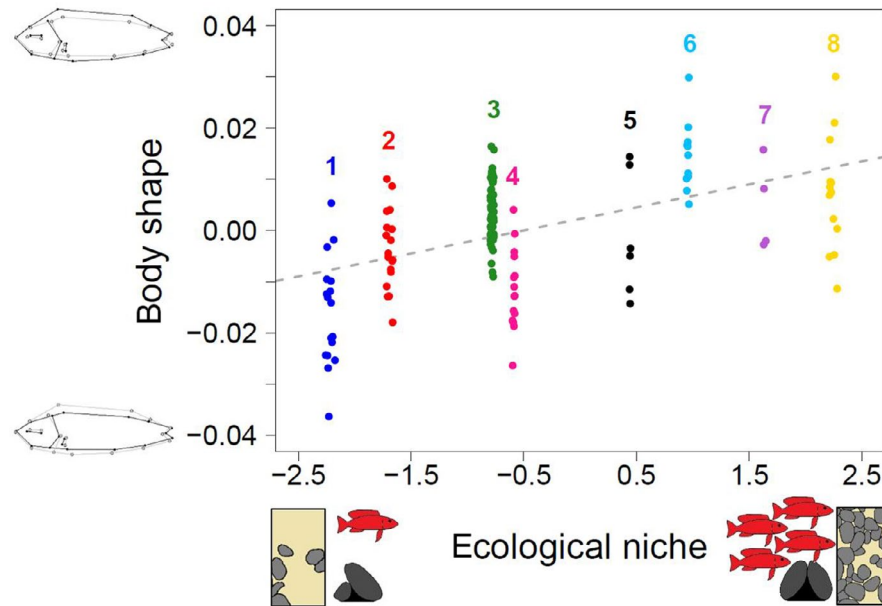


FIGURE 2 Body shape differentiation is correlated with predation pressure and habitat complexity. Shown is the relationship between body shape PC2 of wild-caught fish and the ecological niche as represented by PC scores for each individual. The dashed line denotes the model predicted regression line. The x-axis describes the ecological niche of the population with numbers showing the scores of the ecological PC. The left end describes habitats with low predation risk (red fish), small shelters (space between the two rocks) and high sand cover (rectangles showing yellow sand and grey rocks), that is, low structural complexity. The right end of the axis illustrates habitats with high predation risk, large shelters and low sand cover, that is, high structural complexity. The y-axis represents the body shape scores of individuals, with low scores accounting for shallow bodies and high scores for deep bodies. The black wireframes show the extreme body shapes at both ends of the axis while the grey wireframes denote the score of the opposite end shown as reference

ecological parameters (see Section 3.1), we used the first PC as one-dimensional niche axis in the analysis and refer to it as 'ecological niche' in the following.

2.3 | Data analyses

2.3.1 | Morphological differentiation

We created a set of radiographs of all specimens using a Faxitron LX 60 X-ray device. We excluded specimens that had visible bone fractures leading to deformations of their body shapes (13 wild-caught, 18 F1, 15 F2; see Table 1 for final sample sizes). All observed bone fractures occurred at the skull, except for one individual that had a distorted spine. To quantify the morphology, we used a set of 19 landmarks that were placed on homologous skeletal structures across the body (Figure S1) using the TpsDig 2.3.2 software (Rohlf, 2018).

We used the software R (R Core Team, 2018) with the packages GEOMORPH (Adams et al., 2019) and MORPHO (Schlager, 2017) to perform statistical analyses. First, we performed a generalized Procrustes analysis to correct for size, position and orientation of the specimen on the radiograph. Then, we ran a Procrustes ANOVA with random residual permutation procedure (1,000 iterations) to identify the effects of population and sex on overall body shape of wild-caught fish. We used shape as response variable

and population and sex as explanatory variables. Subsequently, we applied a post-hoc pairwise comparison to identify morphological disparity between populations and estimated its significance by a permutation procedure (1,000 iterations). We conducted a principal component analysis (PCA) to identify the main axes of variance in body shape. PC1 explained 25.2% of the variation and mainly accounted for shape changes at the head and caudal peduncle (Figure S3) known as typical artefacts in fishes. Such changes are not biologically meaningful, as they arise during fixation or result from slight posture differences and are typically captured by PC1s in intraspecific comparisons (i.e. a slight dorsolateral bending of the body; Fruciano, 2016; Laroche et al., 2016; Valentin et al., 2008). PC1 also contained dorsoventral variation in pelvic fin insertion (Figure S3), a signal that likely represents the current condition of the fish (e.g. stomach fullness and spawning condition). Although PC1 also contained subtle signatures of anteroposterior elongation and shortening that might be biologically relevant, these cannot be disentangled from bending and condition artefacts. In contrast, the signal captured in PC2 (explaining 21.9% of the variance; covariation of landmarks describing variation in body depth) was considered informative with respect to the focus of this study, as it mainly captured variation in body depth and elongation (Figure S3). A sharp drop of explained variance occurred after PC2. We therefore proceeded with PC2 as a measure for body shape variation for the following analyses while not considering PC1 to avoid bending and condition artefacts to affect the results. This corresponds to

(UNH154, UNH106 (Lee & Kocher, 1996); NP007, NP773, ULI2 (Schliwen et al., 2001); TmoM11, TmoM13, TmoM25, TmoM27 (Zardoya et al., 1996); Pzeb4 (Van Oppen et al., 1997); UME003 (Parker & Kornfield, 1996); UNH1009 (Carleton et al., 2002); and Ppun21 (Taylor et al., 2002)). Some of these sequences have been optimized for the genome of *N. pulcher* (Kotrschal et al., 2012). Using the QIAGEN® Multiplex PCR Kit, we amplified the DNA allowing co-amplification of several locus-specific, fluorescently labelled primer pairs in one single PCR. We used two different primer sets containing 6 and 7 primer pairs, respectively. PCRs took place in a 10 µl cocktail consisting of 1 µl genomic DNA, 5 µl 2x QIAGEN Multiplex PCR Master Mix, 3 µl H₂O and 1 µl of 10x primer mix. Primer mix contained fluorescent-labelled forward and non-labelled reverse primer pairs with end concentrations of 0.04–0.06 µM each, according to the intensity of the respective amplification products. The fluorescent dyes used were 6-FAM (blue), Yakima Yellow (green), HEX (green), ATTO550 (yellow), ATTO565 (red) (Microsynth), PET (red) and VIC (green) (Thermo Fisher). We amplified the DNA in a GeneAmp® 9700 PCR System (Applied Biosystems) using the following cycling parameters: 15 min at 95°C, 35 cycles at 95°C for 30 s, 57°C for 3 min and 72°C for 60 s followed by a final elongation step of 72°C for 15 min. Fluorescent PCR fragments were visualized by capillary electrophoresis on an ABI 3100® Genetic Analyser (Applied Biosystems). GeneScan 500 LIZ (Thermo Fisher) was used as an internal size standard and the fragments were analysed using GeneMarker® Analysis software version 2.4.0 (SoftGenetics).

We used the software GenAlEx version 6.5 (Peakall & Smouse, 2006, 2012) to conduct genetic analyses. We tested each marker for Hardy–Weinberg equilibrium (HWE) within each population. After correcting for multiple testing by implementing the Bonferroni method (Bland & Altman, 1995), 11 microsatellite markers remained within HWE in each population, which we used for subsequent analyses (Table S2). First, we computed the global and pairwise fixation index (F_{ST}) by running an analysis of molecular variance (AMOVA) with 1,000 permutations to assess significance.

Second, we calculated six standard measures of genetic diversity per population: percentage of polymorphic loci, mean alleles per locus, effective number of alleles, observed and expected heterozygosity, and the fixation index over loci. Third, we performed Bayesian clustering assignment using STRUCTURE version 2.3.4 (Falush et al., 2003, 2007; Hubisz et al., 2009; Pritchard et al., 2000). We performed 10 independent runs for each K between 1 and 10, with a burn-in period of 100,000 iterations and subsequent 100,000 iterations of the Markov Chain Monte Carlo algorithm. We identified optimal cluster numbers using STRUCTURE Harvester (Earl, 2012) following the Evanno method (Evanno et al., 2005). Additionally, we tested whether genetic differentiation is explained by geographical or ecological distance, by running a MRM with genetic distance between populations as response variable, and geographical and ecological distance between populations as explanatory variables. For this purpose, we used the population pairwise distances as explained above.

3 | RESULTS

3.1 | Ecological niche axis

The three ecological parameters of interest (predation risk, shelter size and sand cover) correlated with each other across the eight populations (Spearman correlation, $n = 8$ populations; predation risk–shelter size: $r = 0.83$, $p = 0.015$; predation risk–sand cover: $r = -0.69$, $p = 0.069$; sand cover–shelter size: $r = -0.90$, $p = 0.004$). We therefore combined them into a single, one-dimensional ecological niche axis. This axis accounted for 83.5% of the variation in the natural habitat. Each variable loaded strongly on the PC ($|loading| > 0.52$), where loadings of predation risk and shelter size were positive, and sand cover was negative. The eight populations were distributed continuously across this ecological niche axis (Table 1; Figure 2).

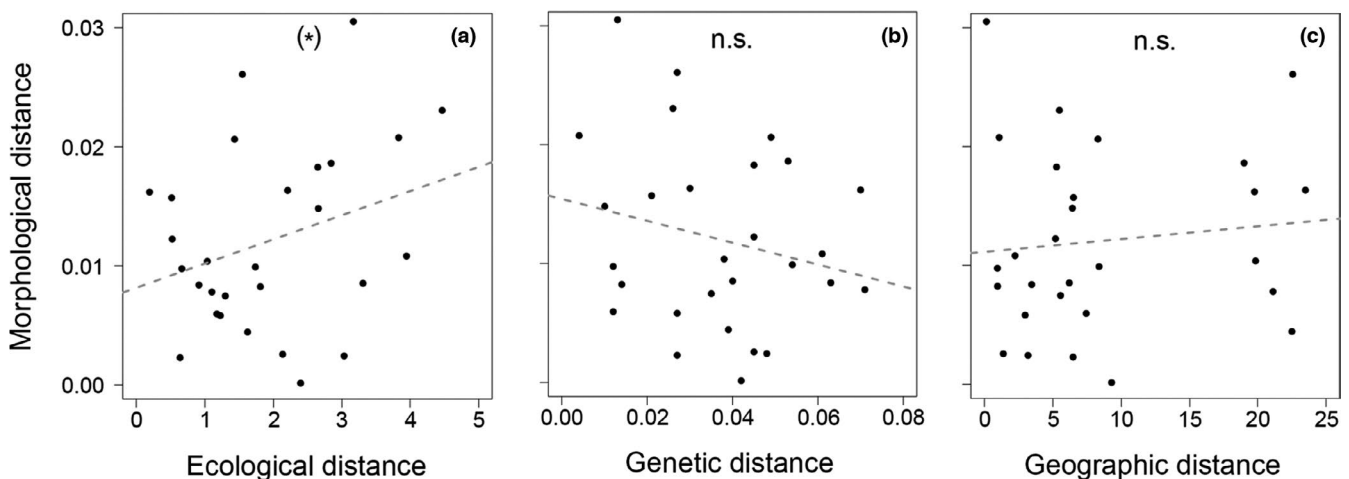


FIGURE 3 Morphological pairwise distance suggests linkage to (a) ecological distance, but neither to (b) genetic nor to (c) geographical distance. The grey dashed lines denote model predicted regression lines. n.s. indicates $p > 0.1$; (*) indicates $p < 0.1$

3.2 | Morphological differentiation

Body shape of wild-caught fish significantly differed between populations (Procrustes ANOVA, $df = 7$, $Z = 9.91$, $p < 0.001$; for post-hoc pairwise comparison see Table S3). Males tended to have a deeper body than females, though this effect was not statistically significant ($df = 1$, $Z = 1.33$, $p = 0.095$). Comparing the overall mean body shape with the means of each population showed that populations 1, 4 and 5 had shallow bodies, while populations 3 and 6 had deep bodies (Figure S4). Populations 4, 6, 7 and 8 showed variation in head and eye size (Figure S4). PC2 accounted for body depth and elongation (Figure S3). Body shape of wild-caught fish was significantly correlated with the ecological niches (LMM, intercept: -0.0002 , $\beta \pm SE$: 0.0047 ± 0.0018 , $df = 1$, $t = 2.59$, $p = 0.014$). Morphological distance between populations tended to show a relationship with ecological distance (MRM, intercept: 0.0095 , β : 0.0022 , $df = 1$, $p = 0.09$; Figure 3a), but neither with genetic distance (β : -0.1157 , $df = 1$, $p = 0.35$; Figure 3b) nor geographical distance (β : 0.0003 , $df = 1$, $p = 0.34$; Figure 3c).

3.3 | Comparison across generations

Average body shape per population of wild-caught fish correlated with that of the F1 generation (LM, intercept: -0.0016 , $\beta \pm SE$: 0.648 ± 0.190 , $df = 1$, $t = 3.41$, $p = 0.013$; Figure 4a). Body shape scores of F1 and F2 fish correlated with each other (LM, intercept: -0.0076 , $\beta \pm SE$: 0.823 ± 0.330 , $df = 1$, $t = 2.49$, $p = 0.027$; Figure 4c), whereas there was no significant correlation of body shape scores between wild-caught and F2 fish (LM, intercept: -0.0091 , $\beta \pm SE$: 0.462 ± 0.323 , $df = 1$, $t = 1.43$, $p = 0.115$; Figure 4b).

3.4 | Genetic differentiation

The AMOVA revealed significant genetic differentiation among populations ($F_{ST} = 0.037$, $p = 0.001$). Population pairwise comparisons revealed that all populations were significantly differentiated from one another, except populations 1 and 7, where we found a non-significant trend (F_{ST} range: 0.004 [populations 1 and 7] to 0.071 [populations 2 and 4]; Table 2b). Microsatellite variation revealed

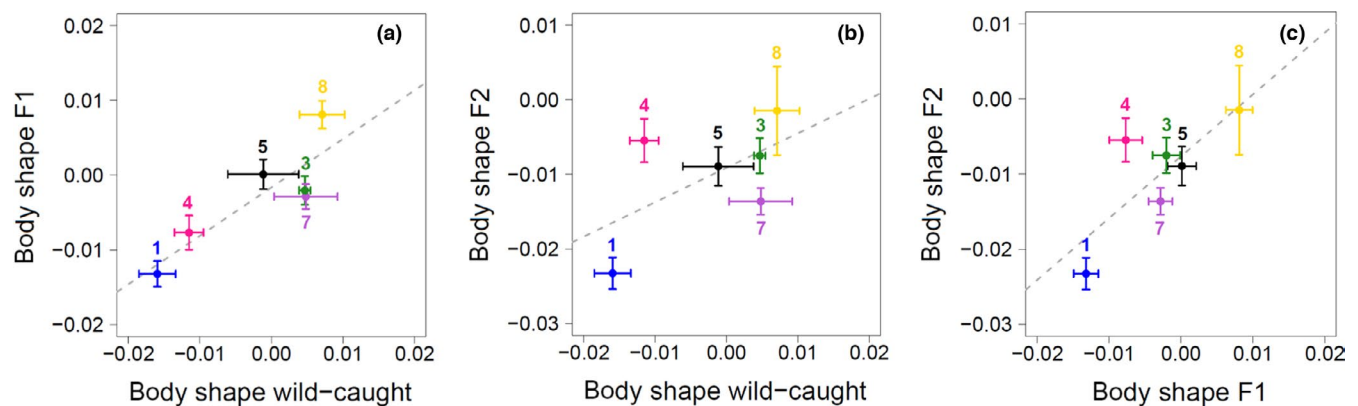


FIGURE 4 Population differentiation in body shape is heritable. (a) Correlation between body shape scores of F1 and wild-caught specimens. (b) Correlation between body shape scores of F2 and wild-caught fish. (c) Correlation between body shape scores of F2 and F1 fish. Shown are means \pm standard errors per population. The dashed lines denote the model predicted regression lines and the populations are colour coded

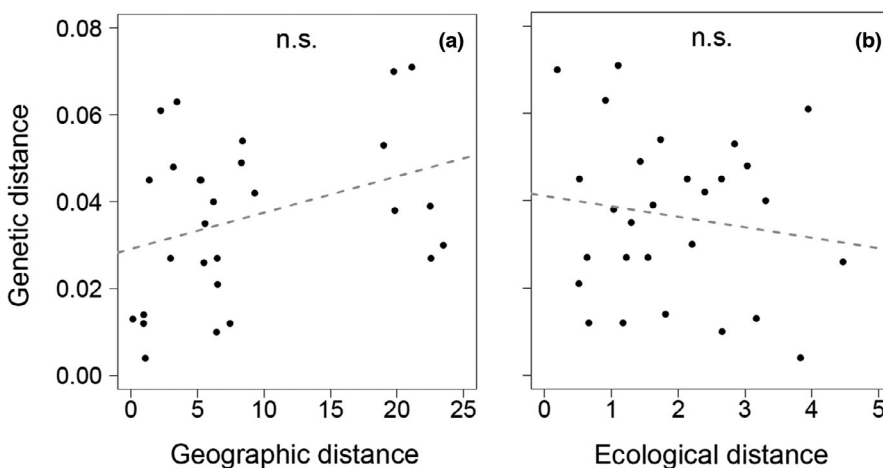


FIGURE 5 Genetic pairwise distance seems independent of (a) geographical and (b) ecological distances. The grey dashed lines denote model predicted regression lines. n.s. indicates $p > 0.1$

moderate levels of allelic diversity and heterozygosity, with no evidence of allele fixation (Table 2a). The STRUCTURE analysis suggested that there are five genetic clusters (see Figure S5 for results for all *K*s calculated). Populations 1, 6 and 7 showed similar allele frequencies, whereas 2, 3, 4 and 8 all had distinct allele compositions and frequencies (Figure 1c). Genetic distance neither correlated with geographical (MRM, intercept: 0.0313, β : 0.0008, $df = 1$, $p = 0.51$; Figure 5a) nor with ecological distance (β : -0.0009, $df = 1$, $p = 0.76$; Figure 5b).

4 | DISCUSSION

Animals can adapt to their environment by changes at different levels, such as social organization, behaviour or morphology. Adaptations to environmental variation at one level potentially constrain or enhance adaptations at other levels, which may lead to a complex interplay of adaptive strategies. Our data revealed significant differentiation between populations linked to their ecological niche in a highly social cichlid. Morphological differentiation between populations was inherited by following generations, suggesting that it reflects heritable adaptation to the local ecology of these fish. We found no link between morphological and neutral genetic differentiation, indicating that morphological differences are not a simple by-product of restricted gene flow and consequential drift.

Body depth significantly increased with ecological niche differences representing enhanced predation risk, high structural complexity and large shelter size (Figure 2). Deep bodies have been predicted to evolve under high risk and high structural complexity, and are expected to be counter-selected in habitats with limited shelter sizes (Brönmark & Miner, 1992; Domenici et al., 2008; Langerhans & Reznick, 2010; Larouche et al., 2020; Nilsson et al., 1995; Ruehl & DeWitt, 2005; Ruehl et al., 2011; Takahashi et al., 2009; Webb, 1982, 1984). The finding that the fish had the deepest bodies in habitats featuring high predation pressure suggests that this may serve as a morphological protection against predation risk, especially if shelter access is not hampered by a deep body. The access to shelters is crucial in *N. pulcher*, which defend territories containing rocky crevasses, excavated chambers underneath stones or empty gastropod shells (Groenewoud et al., 2016; Heg et al., 2005; Taborsky, 2016). Interestingly, the largest differences in body depth were found between populations 1 and 6, which are located only 140 m apart from each other but strongly differ in ecological settings. In population 1, predators are largely absent, shelters are small and large rocks are scarce. In population 6 in contrast, risk of predation is intense, the structural complexity is high and large rocky crevasses and excavated cavities are used as shelters. This shows that morphological differentiation took place on a small spatial scale in the absence of neutral genetic differentiation (cf. Conover et al., 2006). Comparable results have been described for the lamprologine cichlid *Telmatochromis temporalis*, which also inhabits

two ecologically distinct habitats, namely rocky-sandy bottoms and shell beds (Takahashi, 2004; Takahashi et al., 2009). In this species, divergent natural selection apparently induced the evolution of two distinct morphotypes in dependence of their ecological niches. A large morph occurs on rocky-sandy bottoms with large shelters, while a small dwarf morph inhabits the shell beds where shelter sizes are limited (Takahashi et al., 2009). In the present study, available shelter size and predation risk were positively correlated with each other, and hence we cannot disentangle their individual effects on the morphology of *N. pulcher*. To do so, it would be necessary to include populations with small shelter sizes and high predation risk in the analyses. This combination did not appear in any of our study populations, which might indicate that habitats with small shelter sizes and high predation risk may either be less suitable for this species or be scarce in general.

Morphological differentiation tended to increase with increasing ecological differentiation between populations, suggesting that natural selection might lead to body shape differences as a response to habitat variation. In contrast, neither geographical distance nor neutral genetic divergence between populations explained significant amounts of morphological differentiation, corroborating that populations appearing homogeneous for neutral loci may still exhibit local adaptation (Conover et al., 2006). Morphological adaptations to local ecology can either result from phenotypic plasticity or from heritable genetic differences between populations (Bradshaw, 1965; DeWitt & Scheiner, 2004; McCollum & Van Buskirk, 1996; Nosil et al., 2005; Schluter, 2000; Sexton et al., 2014; Slatkin, 1993; Wang & Summers, 2010). We show that the effect of ecology on morphological differentiation in *N. pulcher* persists in two subsequent, common garden reared laboratory generations (Figure 4). Body depth of F1 strongly correlated with body depth of wild-caught fish originating from the same site. Furthermore, body depth of the F2 generation significantly correlated with that of the F1 fish, even if the correlation between F2 and the wild-caught generation was not statistically significant (Figure 4). This finding suggests that shape differences have a heritable component while the exact mechanism of inheritance remains elusive. In addition to changes in the DNA sequence, morphological differences can also be inherited via epigenetic variation, which can be influenced directly by ecological interactions (Anway et al., 2005; Cropley et al., 2006; Fieldes & Amyot, 1999; Richards, 2006; Whitelaw & Whitelaw, 2006), and hence serve as an additional, more dynamic pathway for evolutionary change (Bossdorf et al., 2008). Genome screenings for epigenetic variation are required to check whether this would explain at least part of our results (Bossdorf et al., 2008).

The microsatellite analyses revealed genetic differentiation among populations, indicating limited gene flow. Populations 1, 6 and 7 form one spatial cluster and are also genetically very similar while all other populations are genetically differentiated (Figure 1c). Testing for correlations between genetic, geographical and ecological pairwise distances between these populations revealed that genetic distance neither correlated with geographical

nor with ecological distance (Figure 5). However, the structure plot shows that populations 3 and 4 are genetically as well as geographically isolated from the others, which likely arises by limited dispersal possibilities and prevailing short-distance dispersal (Stiver et al., 2004). Besides geographical distance, dispersal of bottom-dwelling cichlids is also hampered by ecological barriers, such as large muddy or sandy stretches, which do not provide any shelter from predators and are therefore risky to cross (Kobl Müller et al., 2007). Such barriers may account for the genetic differentiation found between the populations 2, 5 and 8, which are genetically distinct although they are located in close proximity to each other (Figure 1). Hence, it is likely the combination of short dispersal distances and unsuitable habitat between populations that leads to limited gene flow. Interestingly, populations 1 and 6 exhibit strong morphological differentiation in the absence of neutral genetic differentiation (Figures 1 and 2), which shows that morphological differentiation is not simply a by-product of restricted gene flow between populations.

A potential alternative explanation for morphological differentiation in the wild-caught fish might be age-related morphological effects (cf. Frommen et al., 2011; Meuthen et al., 2018). We cannot exclude the possibility that the individuals sampled in different populations differed in age, even if only breeder-sized fish were collected, as individuals of some populations (e.g. population 4) were much smaller than those of others (e.g. population 6; Table 1). Age in this species was shown to correlate with body size (Skubic et al., 2004). Additionally, our sampled wild-caught fish were not balanced with respect to sex, which had a weak though non-significant effect on body shape. However, we still found comparable morphological differentiation in the laboratory-reared fish that were similarly aged when sampled and balanced with respect to sex (Table 1). Hence, age and sex differences are unlikely to be responsible for the observed morphological differentiation.

In combination with previously demonstrated differentiation in social structure between the study populations (Groenewoud et al., 2016), our data suggest that this cooperatively breeding vertebrate adapted to its habitat on various levels, which may interact with each other. While morphological adaptations reflect rather slow responses that are difficult to reverse, changes in social structure and behaviour might be more dynamic and adjustable within an individual's lifetime (Gabriel, 2006). Consequently, group structure and social behaviour might be affected both directly by local ecological conditions and indirectly by the impact of morphological adaptations. For example, in *N. pulcher*, subordinates need to show helping behaviour (i.e. territory maintenance and defence, direct brood care) to be allowed to stay in the dominant's territory (Bergmüller & Taborsky, 2005; Fischer et al., 2014; Naef & Taborsky, 2020). The amount and quality of such help might depend not only on the individuals' size (Bruitjes & Taborsky, 2011; Heg & Taborsky, 2010) but also on their morphology, as exemplified by other cooperative breeders (Molet et al., 2012; Simpson et al., 2011; Young & Bennett, 2010). Eventually, differences in morphology might lead to

strict task specialization, as is the case for example in many eusocial insects (Hölldobler & Wilson, 1990).

Morphological differences could eventually have an impact on group structure (Molet et al., 2012; Simpson et al., 2011; Young & Bennett, 2010). For example, among cooperatively breeding fishes, large and deep-bodied helpers might be beneficial under high risk of predation, as they are efficient in antipredator defence and less prone to predation. On the other hand, shallow-bodied individuals may be more vulnerable to predation, and their shallow bodies might be less impressive when performing visual threat displays like spread fins or raised opercula lids (Balzarini et al., 2014). This could lead to a trade-off between the number of deep-bodied helpers versus shallow-bodied helpers in relation to the availability of large shelters in the territory, eventually shifting the predator avoidance strategy from direct defence to hiding in a shelter (Heg & Taborsky, 2010), which may feed back on group structure. In line with this argument, an earlier study on our eight *N. pulcher* populations showed adaptive responses in social structure to ecological differences (Groenewoud et al., 2016), with groups from high-risk populations with large shelters containing more large helpers and groups from low-risk populations with small shelters having less and/or smaller helpers. An intriguing task for future studies would be to elucidate how cooperatively breeding species adjust their negotiations about group memberships and help (Quiñones et al., 2016) in dependence of the local ecology and the resulting morphological differences between populations.

In summary, this study highlights that in a cooperatively breeding vertebrate morphological attributes show adaptation to their local ecological niche. This coincides with systematic variation in social structure and behaviour, which may interact among one another. Clarifying the importance of morphological adaptation to local ecological conditions for the evolution of behaviour and social organization is a worthwhile challenge for future studies of highly social animals.

ACKNOWLEDGEMENTS

We are grateful to the late Hirokazu Tanaka for invaluable help in planning the experiment and collecting the field data. The Department of Fisheries, Ministry of Agriculture and Livestock of Zambia gave the permission to conduct this research and provided logistical support. We thank Taylor Banda and Lawrence Makasa for their continuing support of our work, and Celestine and the late Augustin Mwewa and their team for hosting us at Tanganyika Science Lodge. We are grateful to Frank Groenewoud and Fabian Heussler for help in collecting field data, Arne Jungwirth for support during the field work, Danielle Bonfils for help in preparing the genetic samples and Evi Zwygart for maintaining the breeding line. Sarah Schamel, Eva Stinnesbeck and Serkan Wesel helped us in producing and analysing the X-ray pictures, and Niklas Freudiger in sampling the laboratory-reared fish and creating graphics. Our analyses benefited from discussions with Rudy Riesch and Andreas Schild and from thoughtful comments of three anonymous referees. This study was supported by SNF-grants 310030B_138660, 31003A_156152 and

31003A_176174 to M.T., and 31003A_166470 and 310030_185021 to J.G.F. Open Access Funding provided by Universitat Bern.

AUTHORS' CONTRIBUTIONS

T.T., F.H. and J.G.F. conceived the study; J.G.F. coordinated the project; M.T. and J.G.F. planned the breeding line, which was maintained by A.F. and D.J.; A.F., D.J. and J.G.F. planned the data collection; D.J., J.M.F. and J.G.F. collected the data and fish samples in the field; A.F. prepared the F1 and F2 fish for analyses; A.F. and T.T. produced the X-ray pictures; D.J. and A.F. conducted the microsatellite analyses; A.F. analysed the data and was supported by D.J., J.M.F. and D.A.M.; A.F. wrote the manuscript and was supported by all authors; all authors approved the final version of the manuscript.

ETHICAL NOTE

Field data were collected within the framework of a Memorandum of Understanding between the Department of Fisheries, Ministry of Agriculture and Livestock of Zambia and the University of Bern, and followed the regulations of the 'Zambian prevention of cruelty to animals' act. The breeding line was established in accordance with the ASAB/ABS guidelines for the treatment of animals in behavioural research and teaching, and it was done under the control of the Veterinary Service of the Kanton Bern (Facility number BE 4/11).

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.fj6q573v8> (Freudiger et al., 2021).

ORCID

Annika Freudiger  <https://orcid.org/0000-0003-1795-7944>

Dario Josi  <https://orcid.org/0000-0003-4543-4438>

Jana M. Flury  <https://orcid.org/0000-0001-9994-5490>

David A. Marques  <https://orcid.org/0000-0003-4590-4575>

Michael Taborsky  <https://orcid.org/0000-0002-1357-4316>

Joachim G. Frommen  <https://orcid.org/0000-0002-1752-6944>

REFERENCES

- Adams, D. C., Collyer, M. L., & Kaliontzopoulou, A. (2019). *Geomorph: Software for geometric morphometric analyses*. R package version 3.1.0.
- Agrawal, A. A., Laforsch, C., & Tollrian, R. (1999). Transgenerational induction of defences in animals and plants. *Nature*, 401, 60–63. <https://doi.org/10.1038/43425>
- Albert, A. Y. K., Sawaya, S., Vines, T. H., Knecht, A. K., Miller, C. T., Summers, B. R., & Schluter, D. (2008). The genetics of adaptive shape shift in stickleback: Pleiotropy and effect size. *Evolution*, 62, 76–85. <https://doi.org/10.1111/j.1558-5646.2007.00259.x>
- Anway, M. D., Cupp, A. S., Uzumcu, M., & Skinner, M. K. (2005). Epigenetic transgenerational actions of endocrine disruptors. *Science*, 308, 1466–1469. <https://doi.org/10.1016/j.reprotox.2010.10.012>
- Arnold, K. E., & Owens, I. P. F. (1999). Cooperative breeding in birds: The role of ecology. *Behavioral Ecology*, 10, 465–471. <https://doi.org/10.1093/beheco/10.5.465>
- Balshine, S., Leach, B., Neat, F., Reid, H., Taborsky, M., & Werner, N. (2001). Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). *Behavioral Ecology and Sociobiology*, 50, 134–140. <https://doi.org/10.1007/s002650100343>
- Balshine-Earn, S., Neat, F. C., Reid, H., & Taborsky, M. (1998). Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. *Behavioral Ecology*, 9, 432–438. <https://doi.org/10.1093/beheco/9.5.432>
- Balzarini, V., Taborsky, M., Wanner, S., Koch, F., & Frommen, J. G. (2014). Mirror, mirror on the wall: The predictive value of mirror tests for measuring aggression in fish. *Behavioral Ecology and Sociobiology*, 68, 871–878. <https://doi.org/10.1007/s00265-014-1698-7>
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society*, 57, 289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>
- Bergmüller, R., Heg, D., & Taborsky, M. (2005). Helpers in a cooperatively breeding cichlid stay and pay or disperse and breed, depending on ecological constraints. *Proceedings of the Royal Society B: Biological Sciences*, 272, 325–331. <https://doi.org/10.1098/rspb.2004.2960>
- Bergmüller, R., & Taborsky, M. (2005). Experimental manipulation of helping in a cooperative breeder: Helpers 'pay to stay' by pre-emptive appeasement. *Animal Behaviour*, 69, 19–28. <https://doi.org/10.1016/j.anbehav.2004.05.009>
- Bland, J. M., & Altman, D. G. (1995). Multiple significance tests: The Bonferroni method. *BMJ*, 310, 170. <https://doi.org/10.1136/bmj.310.6973.170>
- Bolnick, D. I., & Otto, S. P. (2013). The magnitude of local adaptation under genotype-dependent dispersal. *Ecology and Evolution*, 3, 4722–4735. <https://doi.org/10.1002/ece3.850>
- Bossdorf, O., Richards, C. L., & Pigliucci, M. (2008). Epigenetics for ecologists. *Ecology Letters*, 11, 106–115. <https://doi.org/10.1111/j.1461-0248.2007.01130.x>
- Bradshaw, A. D. (1965). Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, 13, 115–155. [https://doi.org/10.1016/S0065-2660\(08\)60048-6](https://doi.org/10.1016/S0065-2660(08)60048-6)
- Brönmark, C., & Miner, J. G. (1992). Predator-induced phenotypic change in body morphology in crucian carp. *Science*, 258, 1348–1350. <https://doi.org/10.1126/science.258.5086.1348>
- Bruintjes, R., & Taborsky, M. (2011). Size-dependent task specialization in a cooperative cichlid in response to experimental variation of demand. *Animal Behaviour*, 81, 387–394. <https://doi.org/10.1016/j.anbehav.2010.10.004>
- Cannizzo, Z. J., Nix, S. K., Whaling, I. C., & Griffen, B. D. (2019). Individual morphology and habitat structure alter social interactions in a range-shifting species. *Diversity*, 11, 6. <https://doi.org/10.3390/d11010006>
- Cantor, M., Maldonado-Chaparro, A. A., Beck, K. B., Brandl, H. B., Carter, G. G., He, P., Hillemann, F., Klarevas-Irby, J. A., Ogino, M., Papageorgiou, D., Prox, L., & Farine, D. R. (2020). The importance of individual-to-society feedbacks in animal ecology and evolution. *Journal of Animal Ecology*, 50, 215–222. <https://doi.org/10.1111/1365-2656.13336>
- Carleton, K. L., Streelman, J. T., Lee, B. Y., Garnhart, N., Kidd, M., & Kocher, T. D. (2002). Rapid isolation of CA microsatellites from the tilapia genome. *Animal Genetics*, 33, 140–144. <https://doi.org/10.1046/j.1365-2052.2002.00817.x>
- Conover, D. O., Clarke, L. M., Munch, S. B., & Wagner, G. N. (2006). Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. *Journal of Fish Biology*, 69, 21–47. <https://doi.org/10.1111/j.1095-8649.2006.01274.x>
- Cropley, J. E., Suter, C. M., Beckman, K. B., & Martin, D. I. K. (2006). Germ-line epigenetic modification of the murine Avy allele by nutritional supplementation. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 17308–17312. <https://doi.org/10.1073/pnas.0607090103>

- Curry, R. L. (1989). Geographic variation in social organization of Galápagos mockingbirds: Ecological correlates of group territoriality and cooperative breeding. *Behavioral Ecology and Sociobiology*, 24, 147–160. <https://doi.org/10.1007/BF00302932>
- DeWitt, T. J. (1998). Costs and limits of phenotypic plasticity: Tests with predator-induced morphology and life history in a freshwater snail. *Journal of Evolutionary Biology*, 11, 465–480. <https://doi.org/10.1007/s000360050100>
- DeWitt, T. J., & Scheiner, S. M. (2004). *Phenotypic plasticity: Functional and conceptual approaches*. Oxford University Press.
- DeWitt, T. J., Sih, A., & Hucko, J. A. (1999). Trait compensation and co-specialization in a freshwater snail: Size, shape and antipredator behaviour. *Animal Behaviour*, 58, 397–407. <https://doi.org/10.1006/anbe.1999.1158>
- Domenici, P., Turesson, H., Brodersen, J., & Brönmark, C. (2008). Predator-induced morphology enhances escape locomotion in crucian carp. *Proceedings of the Royal Society B: Biological Sciences*, 275, 195–201. <https://doi.org/10.1098/rspb.2007.1088>
- Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A web-site and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetic Resources*, 4, 359–361. <https://doi.org/10.1007/s12686-011-9548-7>
- Eklöv, P., & Jonsson, P. (2007). Pike predators induce morphological changes in young perch and roach. *Journal of Fish Biology*, 70, 155–164. <https://doi.org/10.1111/j.1095-8649.2006.01283.x>
- Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, 14, 2611–2620. <https://doi.org/10.1111/j.1365-294X.2005.02553.x>
- Falconer, D. S. (1981). *Introduction to quantitative genetics* (2nd ed.). Longman.
- Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, 158, 1567–1587. <https://doi.org/10.1093/genetics/164.4.1567>
- Falush, D., Stephens, M., & Pritchard, J. K. (2007). Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Molecular Ecology Notes*, 7, 574–578. <https://doi.org/10.1111/j.1471-8286.2007.01758.x>
- Fieldes, M. A., & Amyot, L. M. (1999). Epigenetic control of early flowering in flax lines induced by 5-azacytidine applied to germinating seed. *Journal of Heredity*, 90, 199–206. <https://doi.org/10.1093/jhered/90.1.199>
- Fischer, S., Zöttl, M., Groenewoud, F., & Taborsky, B. (2014). Group-size-dependent punishment of idle subordinates in a cooperative breeder where helpers pay to stay. *Proceedings of the Royal Society B: Biological Sciences*, 281, 20140184. <https://doi.org/10.1098/rspb.2014.0184>
- Freudiger, A., Josi, D., Thünken, T., Herder, F., Flury, J. M., Marques, D. A., Taborsky, M., & Frommen, J. G. (2021). Data from: Ecological variation drives morphological differentiation in a highly social vertebrate. *Dryad Digital Repository*, <https://doi.org/10.5061/dryad.fj6q573v8>
- Frommen, J. G., Herder, F., Engqvist, L., Mehli, M., Bakker, T. C. M., Schwarzer, J., & Thünken, T. (2011). Costly plastic morphological responses to predator specific odour cues in three-spined sticklebacks (*Gasterosteus aculeatus*). *Evolutionary Ecology*, 25, 641–656. <https://doi.org/10.1007/s10682-010-9454-6>
- Fruciano, C. (2016). Measurement error in geometric morphometrics. *Development Genes and Evolution*, 226, 139–158. <https://doi.org/10.1007/s00427-016-0537-4>
- Gabriel, W. (2006). Selective advantage of irreversible and reversible phenotypic plasticity. *Archiv für Hydrobiologie*, 167, 1–20. <https://doi.org/10.1127/0003-9136/2006/0167-0001>
- Gashagaza, M. M., Nakaya, K., & Sato, T. (1995). Taxonomy of small-sized cichlid fishes in the shell-bed area of Lake Tanganyika. *Japanese Journal of Ichthyology*, 53, 1689–1699. <https://doi.org/10.1017/CBO9781107415324.004>
- Ghalambor, C. K., Reznick, D. N., & Walker, J. A. (2004). Constraints on adaptive evolution: The functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). *The American Naturalist*, 164, 38–50. <https://doi.org/10.1086/421412>
- Godin, J.-G. J. (1997). *Behavioural ecology of teleost fishes*. Oxford University Press.
- Goslee, S. C., & Urban, D. L. (2007). The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*, 22, 1–19.
- Groenewoud, F., Frommen, J. G., Josi, D., Tanaka, H., Jungwirth, A., & Taborsky, M. (2016). Predation risk drives social complexity in cooperative breeders. *Proceedings of the National Academy of Sciences of the United States of America*, 113, 4104–4109. <https://doi.org/10.1073/pnas.1524178113>
- Hara, T. J., & Pitcher, T. J. (1986). *Behaviour of teleost fishes*. Croom Helm.
- Heg, D., Brouwer, L., Bachar, Z., & Taborsky, M. (2005). Large group size yields group stability in the cooperatively breeding cichlid *Neolamprologus pulcher*. *Behaviour*, 142, 1615–1641. <https://doi.org/10.1163/156853905774831891>
- Heg, D., & Taborsky, M. (2010). Helper response to experimentally manipulated predation risk in the cooperatively breeding cichlid *Neolamprologus pulcher*. *PLoS ONE*, 5, e10784. <https://doi.org/10.1371/journal.pone.0010784>
- Hereford, J. (2009). A quantitative survey of local adaptation and fitness trade-offs. *The American Naturalist*, 173, 579–588. <https://doi.org/10.1086/597611>
- Heynen, M., Bunnefeld, N., & Borchering, J. (2017). Facing different predators: Adaptiveness of behavioral and morphological traits under predation. *Current Zoology*, 63, 249–257. <https://doi.org/10.1093/cz/zow056>
- Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Harvard University Press.
- Hubisz, M. J., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, 9, 1322–1332. <https://doi.org/10.1111/j.1755-0998.2009.02591.x>
- Hudson, A. G., Vonlanthen, P., Bezault, E., & Seehausen, O. (2013). Genomic signatures of relaxed disruptive selection associated with speciation reversal in whitefish. *BMC Evolutionary Biology*, 13, 1–18. <https://doi.org/10.1186/1471-2148-13-108>
- Hüllen, S., Mandl, C., Geiger, M. F., Hadiaty, R. K., Wahyudewantoro, G., Ziegler, T., & Herder, F. (2021). On the challenge to correctly identify Rasboras (Teleostei: Cyprinidae: Danioninae) inhabiting the Mesangat Wetlands, East Kalimantan, Indonesia. *Diversity*, 13, 8. <https://doi.org/10.3390/d13010008>
- Imre, I., McLaughlin, R. L., & Noakes, D. L. G. (2002). Phenotypic plasticity in brook charr: Changes in caudal fin induced by water flow. *Journal of Fish Biology*, 61, 1171–1181. <https://doi.org/10.1006/jfbi.2002.2131>
- Josi, D., Taborsky, M., & Frommen, J. G. (2018). Habitat preferences depend on substrate quality in a cooperative breeder. *Evolutionary Ecology Research*, 19, 517–527. <https://doi.org/10.7892/boris.124601>
- Josi, D., Taborsky, M., & Frommen, J. G. (2019). First field evidence for alloparental egg care in cooperatively breeding fish. *Ethology*, 125, 164–169. <https://doi.org/10.1111/eth.12838>
- Jungwirth, A., Josi, D., Walker, J., & Taborsky, M. (2015). Benefits of coloniality: Communal defence saves anti-predator effort in cooperative breeders. *Functional Ecology*, 29, 1218–1224. <https://doi.org/10.1111/1365-2435.12430>
- Kobl Müller, S., Sefc, K. M., Duftner, N., Warum, M., & Sturmbauer, C. (2007). Genetic population structure as indirect measure of dispersal ability in a Lake Tanganyika cichlid. *Genetica*, 130, 121–131. <https://doi.org/10.1007/s10709-006-0027-0>

- Koenig, W. D. (1981). Reproductive success, group size, and the evolution of cooperative breeding in the Acorn Woodpecker. *The American Naturalist*, 117, 421–443. <https://doi.org/10.1086/283726>
- Koenig, W. D., Walters, E. L., & Haydock, J. (2011). Variable helper effects, ecological conditions, and the evolution of cooperative breeding in the Acorn Woodpecker. *The American Naturalist*, 178, 145–158. <https://doi.org/10.1086/660832>
- Kotrschal, A., Heckel, G., Bonfils, D., & Taborsky, B. (2012). Life-stage specific environments in a cichlid fish: Implications for inducible maternal effects. *Evolutionary Ecology*, 26, 123–137. <https://doi.org/10.1007/s10682-011-9495-5>
- Krueger, D. A., & Dodson, S. I. (1981). Embryological induction and predation ecology in *Daphnia pulex*. *Limnology and Oceanography*, 26, 219–223. <https://doi.org/10.4319/lo.1981.26.2.0219>
- Langerhans, R. B. (2009). Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. *Journal of Evolutionary Biology*, 22, 1057–1075. <https://doi.org/10.1111/j.1420-9101.2009.01716.x>
- Langerhans, R. B., & Reznick, D. N. (2010). Ecology and evolution of swimming performance in fishes: Predicting evolution with biomechanics. In P. Domenici & B. G. Kapoor (Eds.), *Fish locomotion: An ecological perspective* (pp. 200–248). Science Publishers. <https://doi.org/10.1201/b10190>
- Larochelle, C. R., Pickens, F. A. T., Burns, M. D., & Sidlauskas, B. L. (2016). Long-term isopropanol storage does not alter fish morphometrics. *Copeia*, 104, 411–420. <https://doi.org/10.1643/CG-15-303>
- Larouche, O., Benton, B., Corn, K. A., Friedman, S. T., Gross, D., Iwan, M., Kessler, B., Martinez, C. M., Rodriguez, S., Whelpley, H., Wainwright, P. C., & Price, S. A. (2020). Reef-associated fishes have more maneuverable body shapes at a macroevolutionary scale. *Coral Reefs*, 39, 1427–1439. <https://doi.org/10.1007/s00338-020-01976-w>
- Lee, W. J., & Kocher, T. D. (1996). Microsatellite DNA markers for genetic mapping in *Oreochromis niloticus*. *Journal of Fish Biology*, 49, 169–171. <https://doi.org/10.1006/jfbi.1996.0145>
- Marshall, C. A., & Wund, M. A. (2017). The evolution of correlations between behavioural and morphological defence in Alaskan threespine stickleback fish (*Gasterosteus aculeatus*): Evidence for trait compensation and co-specialization. *Evolutionary Ecology Research*, 18, 305–322.
- Marshall, H. H., Sanderson, J. L., Mwanghuya, F., Businge, R., Kyabulima, S., Hares, M. C., Inzani, E., Kalema-Zikusoka, G., Mwesige, K., Thompson, F. J., Vitikainen, E. I. K., & Cant, M. A. (2016). Variable ecological conditions promote male helping by changing banded mongoose group composition. *Behavioral Ecology*, 27, 978–987. <https://doi.org/10.1093/beheco/aww006>
- McCollum, S. A., & Van Buskirk, J. (1996). Costs and benefits of a predator-induced polyphenism in the Gray Treefrog *Hyla chrysoscelis*. *Evolution*, 50, 583–593. <https://doi.org/10.2307/2410833>
- Meuthen, D., Baldauf, S. A., Bakker, T. C. M., & Thünken, T. (2018). Neglected patterns of variation in phenotypic plasticity: Age- and sex-specific antipredator plasticity in a cichlid fish. *The American Naturalist*, 191, 475–490. <https://doi.org/10.1086/696264>
- Miner, B. G., Sultan, S. E., Morgan, S. G., Padilla, D. K., & Relyea, R. A. (2005). Ecological consequences of phenotypic plasticity. *Trends in Ecology & Evolution*, 20, 685–692. <https://doi.org/10.1016/j.tree.2005.08.002>
- Molet, M., Wheeler, D. E., & Peeters, C. (2012). Evolution of novel mosaic castes in ants: Modularity, phenotypic plasticity, and colonial buffering. *The American Naturalist*, 180, 328–341. <https://doi.org/10.1086/667368>
- Mushagalusa, D. C., Awata, S., Satoh, S., Ota, K., Hori, M., Nshombo, M., & Kohda, M. (2019). Do scales of the cichlid *Altolamprologus compressiceps* in Lake Tanganyika function as a morphological defense against scale-eating? *Zoological Science*, 36, 147–153. <https://doi.org/10.2108/zs180130>
- Naef, J., & Taborsky, M. (2020). Commodity-specific punishment for experimentally induced defection in cooperatively breeding fish. *Royal Society Open Science*, 7, 191808. <https://doi.org/10.1098/rsos.191808>
- Nilsson, P. A., & Brönmark, C. (2000). Prey vulnerability to a gape-size limited predator: Behavioural and morphological impacts on northern pike piscivory. *Oikos*, 88, 539–546. <https://doi.org/10.1034/j.1600-0706.2000.880310.x>
- Nilsson, P. A., Brönmark, C., & Pettersson, L. B. (1995). Benefits of a predator-induced morphology in crucian carp. *Oecologia*, 104, 291–296. <https://doi.org/10.1007/BF00328363>
- Nosil, P., Villoutreix, R., De Carvalho, C. F., Farkas, T. E., Soria-Carrasco, V., Feder, J. L., Crespi, B. J., & Gompert, Z. (2018). Natural selection and the predictability of evolution in *Timema* stick insects. *Science*, 359, 765–770. <https://doi.org/10.1126/science.aap9125>
- Nosil, P., Vines, T. H., & Funk, D. J. (2005). Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution*, 59, 705–719. <https://doi.org/10.1111/j.0014-3820.2005.tb01747.x>
- Olsson, J., & Eklöv, P. (2005). Habitat structure, feeding mode and morphological reversibility: Factors influencing phenotypic plasticity in perch. *Evolutionary Ecology Research*, 7, 1109–1123.
- Parker, A., & Kornfield, I. (1996). Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Environmental Biology of Fishes*, 47, 345–352. <https://doi.org/10.1007/BF00005049>
- Peakall, R., & Smouse, P. E. (2006). GenAIX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6, 288–295. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Peakall, R., & Smouse, P. E. (2012). GenAIX 6.5: Genetic analysis in Excel. Population genetic software for teaching and research – An update. *Bioinformatics*, 28, 2537–2539. <https://doi.org/10.1093/bioinformatics/bts460>
- Popma, T., & Masser, M. (1999). *Tilapia life history and biology*. Southern Regional Aquaculture Center.
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959. <https://doi.org/10.1093/genetics/155.2.945>
- Quiñones, A. E., Van Doorn, G. S., Pen, I., Weissing, F. J., & Taborsky, M. (2016). Negotiation and appeasement can be more effective drivers of sociality than kin selection. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371, 20150089. <https://doi.org/10.1098/rstb.2015.0089>
- R Core Team. (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from <https://www.r-project.org/>
- Richards, E. J. (2006). Revisiting soft inheritance. *Nature Review Genetics*, 7, 395–402. <https://doi.org/10.1038/nrg1834>
- Riesch, R., Martin, R. A., Diamond, S. E., Jourdan, J., Plath, M., & Langerhans, R. B. (2018). Thermal regime drives a latitudinal gradient in morphology and life history in a livebearing fish. *Biological Journal of the Linnean Society*, 125, 126–141. <https://doi.org/10.1093/BLOI/NNEAN/BLY095>
- Rohlf, F. J. (2018). *TpsDig, version 2.3.2*. Stony Brook: Department of Ecology and Evolution, State University of New York.
- Rohner, P. T., & Moczek, A. P. (2020). Rapid differentiation of plasticity in life history and morphology during invasive range expansion and concurrent local adaptation in the horned beetle *Onthophagus taurus*. *Evolution*, 74, 2059–2072. <https://doi.org/10.1111/evo.14045>
- Ruehl, C. B., & DeWitt, T. J. (2005). Trophic plasticity and fine-grained resource variation in populations of western mosquitofish, *Gambusia affinis*. *Evolutionary Ecology Research*, 7, 801–819.
- Ruehl, C. B., Shervette, V., & Dewitt, T. J. (2011). Replicated shape variation between simple and complex habitats in two estuarine fishes.

- Biological Journal of the Linnean Society, 103, 147–158. <https://doi.org/10.1111/j.1095-8312.2011.01626.x>
- Schlager, S. (2017). Morpho and Rvcg – Shape analysis in R. In G. Zheng, S. Li, & G. Székely (Eds.), *Statistical shape and deformation analysis* (pp. 217–256). Academic Press.
- Schliwen, U., Rassmann, K., Markmann, M., Markert, J., Kocher, T., & Tautz, D. (2001). Genetic and ecological divergence of a monophyletic cichlid species pair under fully sympatric conditions in Lake Ejagham, Cameroon. *Molecular Ecology*, 10, 1471–1488. <https://doi.org/10.1046/j.1365-294X.2001.01276.x>
- Schluter, D. (2000). *The ecology of adaptive radiation*. Oxford University Press.
- Schrank, A. J., Webb, P. W., & Mayberry, S. (1999). How do body and paired-fin positions affect the ability of three teleost fishes to maneuver around bends? *Canadian Journal of Zoology*, 77, 203–210. <https://doi.org/10.1139/z98-209>
- Sexton, J. P., Hangartner, S. B., & Hoffmann, A. A. (2014). Genetic isolation by environment or distance: Which pattern of gene flow is most common? *Evolution*, 68, 1–15. <https://doi.org/10.1111/evo.12258>
- Simpson, S. J., Sword, G. A., & Lo, N. (2011). Polyphenism in insects. *Current Biology*, 21, R738–R749. <https://doi.org/10.1016/j.cub.2011.06.006>
- Skubic, E., Taborsky, M., McNamara, J. M., & Houston, A. I. (2004). When to parasitize? A dynamic optimization model of reproductive strategies in a cooperative breeder. *Journal of Theoretical Biology*, 227, 487–501. <https://doi.org/10.1016/j.jtbi.2003.11.021>
- Slatkin, M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, 47, 264–279. <https://doi.org/10.1111/j.1558-5646.1993.tb01215.x>
- Stauffer, J. R., & van Snik Gray, E. (2004). Phenotypic plasticity: Its role in trophic radiation and explosive speciation in cichlids (Teleostei: Cichlidae). *Animal Biology*, 54, 137–158. <https://doi.org/10.1163/1570756041445191>
- Stiver, K. A., Desjardins, J. K., Fitzpatrick, J. L., Neff, B., Quinn, J. S., & Balshine, S. (2007). Evidence for size and sex-specific dispersal in a cooperatively breeding cichlid fish. *Molecular Ecology*, 16, 2974–2984. <https://doi.org/10.1111/j.1365-294X.2007.03350.x>
- Stiver, K. A., Dierkes, P., Taborsky, M., & Balshine, S. (2004). Dispersal patterns and status change in a cooperatively breeding cichlid *Neolamprologus pulcher*: Evidence from microsatellite analyses and behavioural observations. *Journal of Fish Biology*, 65, 91–105. <https://doi.org/10.1111/j.0022-1112.2004.00427.x>
- Taborsky, M. (1985). Breeder-helper conflict in a cichlid fish with brood-care helpers: An experimental analysis. *Behaviour*, 95, 45–75. <https://doi.org/10.1163/156853985X00046>
- Taborsky, M. (2016). Cichlid fishes: A model for the integrative study of social behavior. In W. D. Koenig, & J. L. Dickinson (Eds.), *Cooperative breeding in vertebrates: Studies of ecology, evolution, and behaviour* (pp. 272–293). Cambridge University Press. <https://doi.org/10.1017/CBO9781107338357.017>
- Taborsky, M., & Wong, M. (2017). Sociality in fishes. In D. R. Rubenstein & P. Abbot (Eds.), *Comparative social evolution* (pp. 354–389). Cambridge University Press.
- Takahashi, T. (2004). Morphological and genetic distinctness of rock and shell-bed dwelling *Telmatochromis* (Teleostei, Cichlidae) in the south of Lake Tanganyika suggest the existence of two species. *Journal of Fish Biology*, 65, 419–435. <https://doi.org/10.1111/j.0022-1112.2004.00459.x>
- Takahashi, T., Watanabe, K., Munehara, H., Rüber, L., & Hori, M. (2009). Evidence for divergent natural selection of a Lake Tanganyika cichlid inferred from repeated radiations in body size. *Molecular Ecology*, 18, 3110–3119. <https://doi.org/10.1111/j.1365-294X.2009.04248.x>
- Tanaka, H., Frommen, J. G., Takahashi, T., & Kohda, M. (2016). Predation risk promotes delayed dispersal in the cooperatively breeding cichlid *Neolamprologus obscurus*. *Animal Behaviour*, 117, 51–58. <https://doi.org/10.1016/j.anbehav.2016.04.019>
- Taylor, E. B., & McPhail, J. D. (1985). Variation in body morphology among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. *Canadian Journal of Zoology*, 42, 2020–2028. <https://doi.org/10.1139/f85-249>
- Taylor, E. B., & McPhail, J. D. (1986). Prolonged and burst swimming in anadromous and freshwater threespine stickleback, *Gasterosteus aculeatus*. *Canadian Journal of Zoology*, 64, 416–420. <https://doi.org/10.1139/z86-064>
- 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* × *Pundamilia nyererei* hybrid. *Molecular Ecology Notes*, 2, 443–445. <https://doi.org/10.1046/j.1471-8286.2002.00272.x>
- Valentin, A. E., Penin, X., Chanut, J. P., Sévigny, J. M., & Rohlf, F. J. (2008). Arching effect on fish body shape in geometric morphometric studies. *Journal of Fish Biology*, 73, 623–638. <https://doi.org/10.1111/j.1095-8649.2008.01961.x>
- Van Oppen, M. J. H., Rico, C., Deutsch, J. C., Turner, G. F., & Hewitt, G. M. (1997). Isolation and characterization of microsatellite loci in the cichlid fish *Pseudotropheus zebra*. *Molecular Ecology*, 6, 387–388. <https://doi.org/10.1046/j.1365-294X.1997.00188.x>
- Walker, J. A. (1997). Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biological Journal of the Linnean Society*, 61, 3–50. <https://doi.org/10.1006/bijl.1996.9999>
- Wang, I. J., & Summers, K. (2010). Genetic structure is correlated with phenotypic divergence rather than geographic isolation in the highly polymorphic strawberry poison-dart frog. *Molecular Ecology*, 19, 447–458. <https://doi.org/10.1111/j.1365-294X.2009.04465.x>
- Webb, P. W. (1982). Locomotor patterns in the evolution of actinopterygian fishes. *American Zoologist*, 22, 329–342. <https://doi.org/10.1093/icb/22.2.329>
- Webb, P. W. (1984). Body form, locomotion and foraging in aquatic vertebrates. *The American Naturalist*, 24, 107–120. <https://doi.org/10.1093/icb/24.1.107>
- Webb, P. W., LaLiberte, G. D., & Schrank, A. J. (1996). Does body and fin form affect the maneuverability of fish traversing vertical and horizontal slits? *Environmental Biology of Fishes*, 46, 7–14. <https://doi.org/10.1007/BF00001692>
- Webb, P. W., & Weihs, D. (1986). Functional locomotor morphology of early life history stages of fishes. *Transactions of the American Fisheries Society*, 115, 115–127. [https://doi.org/10.1577/1548-8659\(1986\)115<115:flmoel>2.0.co;2](https://doi.org/10.1577/1548-8659(1986)115<115:flmoel>2.0.co;2)
- Weber, M. J., Rounds, K. D., & Brown, M. L. (2012). Phenotypic variation and associated predation risk of juvenile common carp *Cyprinus carpio*. *Journal of Fish Biology*, 80, 49–60. <https://doi.org/10.1111/j.1095-8649.2011.03140.x>
- West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics*, 20, 249–278. <https://doi.org/10.1146/annurev.es.20.110189.001341>
- West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.
- Whitelaw, N. C., & Whitelaw, E. (2006). How lifetimes shape epigenotype within and across generations. *Human Molecular Genetics*, 15, 131–137. <https://doi.org/10.1093/hmg/ddl200>
- Willmer, P., Stone, G. N., & Johnston, I. (2005). *Environmental physiology of animals*. John Wiley & Sons.
- Wright, S. (1943). Isolation by distance. *Genetics*, 28, 114–138.
- Young, A. J., & Bennett, N. C. (2010). Morphological divergence of breeders and helpers in wild Damaraland mole-rat societies. *Evolution*, 64, 3190–3197. <https://doi.org/10.1111/j.1558-5646.2010.01066.x>
- Young, T. P., Stanton, M. L., & Christian, C. E. (2003). Effects of natural and simulated herbivory on spine lengths of *Acacia*

- drepanolobium* in Kenya. *Oikos*, 101, 171–179. <https://doi.org/10.1034/j.1600-0706.2003.12067.x>
- Zardoya, R., Vollmer, D. M., Craddock, C., Streelman, J. T., Karl, S., & Meyer, A. (1996). Evolutionary conservation of microsatellite flanking regions and their use in resolving the phylogeny of cichlid fishes (Pisces: Perciformes). *Proceedings of the Royal Society B: Biological Sciences*, 263, 1589–1598. <https://doi.org/10.1098/rspb.1996.0233>
- Zöttl, M., Frommen, J. G., & Taborsky, M. (2013). Group size adjustment to ecological demand in a cooperative breeder. *Proceedings of the Royal Society B: Biological Sciences*, 280, 20122772. <https://doi.org/10.1098/rspb.2012.2772>
- Zuk, M., Kim, T., Robinson, S. I., & Johnsen, T. S. (1998). Parasites influence social rank and morphology, but not mate choice, in female red junglefowl, *Gallus gallus*. *Animal Behaviour*, 56, 493–499. <https://doi.org/10.1006/anbe.1998.0807>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Freudiger, A., Josi, D., Thünken, T., Herder, F., Flury, J. M., Marques, D. A., Taborsky, M., & Frommen, J. G. (2021). Ecological variation drives morphological differentiation in a highly social vertebrate. *Functional Ecology*, 35, 2266–2281. <https://doi.org/10.1111/1365-2435.13857>