

Mouth dimorphism in scale-eating cichlid fish from Lake Tanganyika advances individual fitness

Adrian Indermaur,¹ Anya Theis,¹ Bernd Egger,¹ and Walter Salzburger^{1,2}

¹Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland

²E-mail: walter.salzburger@unibas.ch

Received January 4, 2018

Accepted July 4, 2018

Random asymmetry, that is the coexistence of left- and right-sided (or -handed) individuals within a population, is a particular case of natural variation; what triggers and maintains such dimorphisms remains unknown in most cases. Here, we report a field-based cage experiment in the scale-eating Tanganyikan cichlid *Perissodus microlepis*, which occurs in two morphs in nature: left-skewed and right-skewed individuals with respect to mouth orientation. Using underwater cages stocked with scale-eaters and natural prey fish, we first confirm that, under semi-natural conditions, left-skewed scale-eaters preferentially attack the right flank of their prey, whereas right-skewed individuals feed predominantly from the left side. We then demonstrate that scale-eaters have a higher probability for successful attacks when kept in dimorphic experimental populations (left- and right-skewed morphs together) as compared to monomorphic populations (left- or right-skewed morphs), most likely because prey fishes fail to accustom to strikes from both sides. The significantly increased probability for attacks appears to be the selective agent responsible for the evolution and maintenance of mouth dimorphism in *P. microlepis*, lending further support to the hypothesis that negative frequency-dependent selection is the stabilizing force balancing the mouth dimorphism at quasi-equal ratios in scale-eating cichlids.

KEY WORDS: Cage experiment, Cichlidae, ecological specialization, mouth asymmetry, *Perissodus microlepis*.

Variation in morphology between individuals plays a crucial role in the adaptive evolution of natural populations (e.g., Darwin 1859; Nosil 2012). Morphological variation is most often manifested in a symmetrical and continuous trait variance among individuals within populations, but there are also cases where the natural symmetry is broken and morphological asymmetries exist (Palmer 1994, 2004, 2010). In many cases, these are random asymmetries, meaning that both right- and left-sided (or: right- and left-“handed”) individuals occur within a population at certain frequencies, making a population polymorphic; as opposed to dextral and sinistral asymmetries, where only right- or left-sided individuals are present (Van Valen 1962; Palmer 2009, 2010). Examples for random morphological asymmetries are, among others, the claws of American lobsters (about half of the individuals have the larger crusher claw on the right side and the other half on the left) (Govind 1989; Palmer 2005), or the eyes of some flatfish (either the right or the left eye migrates, during ontogeny, to the other, then upside, sphere of the face) (Schreiber 2006; Friedman 2008).

In most cases, the selective regimes maintaining random asymmetries in natural populations are unknown (Palmer 2009, 2010). One candidate mechanism to produce stable polymorphisms in natural populations is negative frequency-dependent selection, whereby the fitness of a particular morph decreases as its frequency increases (Clarke and O’Donald 1964; Ayala and Campbell 1974). Examples of negative frequency dependence include interactions between hosts and parasites (Koskella and Lively 2009), between pollinators and plants (Smithson and Macnair 1997; Gigord et al. 2001), and between predators and prey (Hori 1993), among others.

A fascinating case of random asymmetry is found in several species of scale-eating cichlid fishes endemic to East African Lake Tanganyika, which show an extensive left/right mouth dimorphism and have become a textbook example for behavioral and morphological laterality (Fryer and Iles 1972; Futuyama 2009; Takeuchi et al. 2016) as well as for negative frequency-dependent selection (Hori 1993; Takeuchi et al. 2012). These scale-eaters belong to the Perissodini, a relatively species-poor cichlid

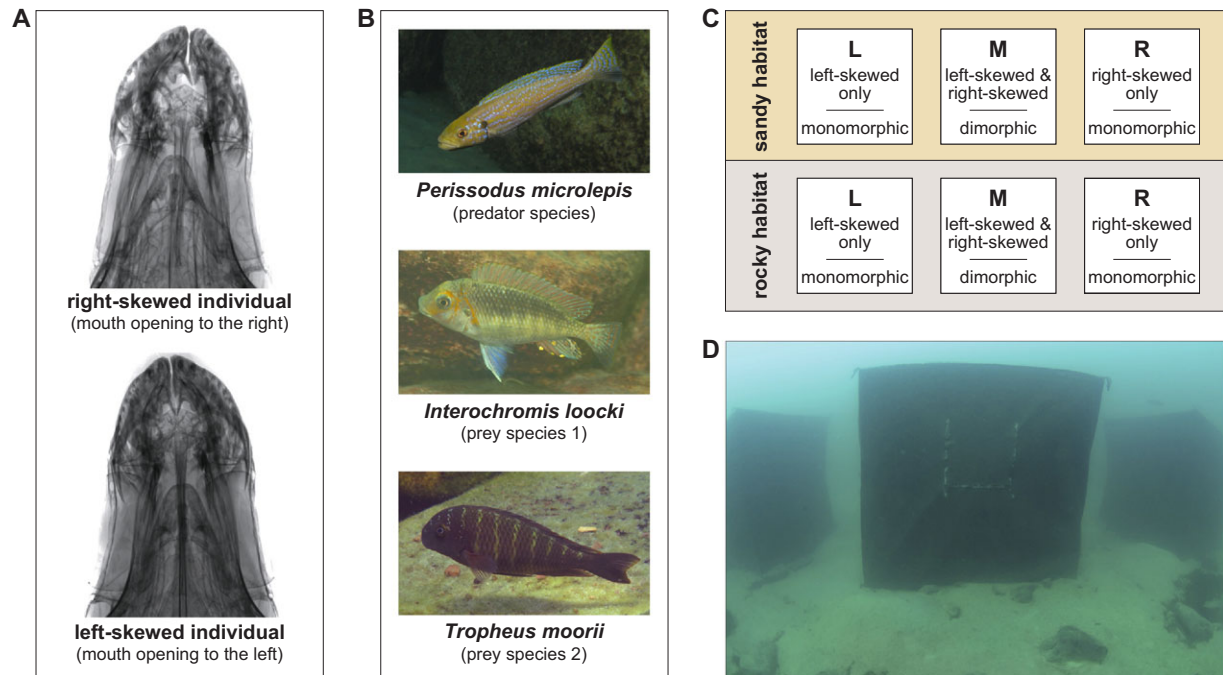


Figure 1. Experimental setup. (A) X-ray images showing the head region of *P. microlepis* with different mouth morphs in dorsal view. Above: “right-skewed” (where the left upper jaw bow is elongated); below: left-skewed (where the right upper jaw bow is elongated) (pictures by Heinz H. Büscher). (B) Underwater photographs of the predator and prey species in their natural habitat (pictures by Adrian Indermaur). (C) Scheme of the experimental setup showing the cages (squares) with the distribution of the different mouth morphs (left-skewed/right-skewed), experimental populations (L/M/R), experimental population setups (mono-/dimorphic), and habitats (rocky/sandy bottom). (Note that the experimental arrangement was randomly rotated within habitat in every trial). (D) Underwater photograph of the experimental cages (picture by Angel M. Fitor).

lineage counting nine described species (Liem and Stewart 1976; Koblmüller et al. 2007). At least six *Perissodini* species exhibit a particularly specialized feeding mode in that they live, to various degrees, on scales and epidermis of other fishes, a strategy known as lepidophagy (Marlier and Leleup 1954; Takahashi et al. 2007a, b). Scale-eating cichlids have evolved remarkable adaptations such as the afore-mentioned asymmetry of mouth opening (Fig. 1A), hook-like teeth (Takahashi et al. 2007a, b), as well as sophisticated attack strategies including aggressive mimicry (Hori and Watanabe 2000; Boileau et al. 2015).

Perissodus microlepis (Boulenger 1898) is the most common and perhaps the most specialized lepidophagous cichlid in Lake Tanganyika, and feeds almost exclusively upon scales of other fishes (Takahashi et al. 2007b; Muschick et al. 2012; Takeuchi et al. 2016). It hunts and feeds by ambushing its prey fish from the rear, instantly attacks the flanks of its victim, and bites out a single or a bunch of scales together with epidermis. For a long time, it has been noted that *P. microlepis* come in two discrete morphs with respect to mouth morphology (Fig. 1A), ones with a mouth opening to the left side (“left-skewed”; the right upper jaw bow is elongated) and ones with a mouth opening to the right side (“right-skewed”; the left upper jaw bow is elongated) (Fryer and Iles 1972; Hori 1993). More recently, however,

it was suggested that the mouth-opening angle has a more continuous distribution in *P. microlepis* (Van Dooren et al. 2010; Kusche et al. 2012), whereas Takeuchi et al. (2016) found that the lower jaw bones of *P. microlepis* show a bimodal distribution. The genetic and developmental underpinnings of the asymmetry of mouth opening in *P. microlepis* remain elusive. While originally assumed a trait controlled by a single Mendelian locus (Hori 1993; Hori et al. 2007; Takahashi and Hori 2008; Stewart and Albertson 2010), evidence is accumulating that mouth asymmetry is in fact a quantitative trait influenced by external factors (see e.g., Van Dooren et al. 2010; Kusche et al. 2012; Lee et al. 2010, 2012, 2015; Raffini et al. 2017; Takeuchi and Oda 2017).

The mouth dimorphism in *P. microlepis* has long been implicated with a lateralized feeding behavior. Hori (1993) observed that individuals with a left-skewed mouth preferentially attack the right flank of prey fish, while individuals with a right-skewed mouth mainly feed from the prey’s left flank. Hori (1993) further showed that natural populations of *P. microlepis* fluctuate around a 50:50 left-to-right-skewed-ratio (with an amplitude of 0.15 and a cycle duration of about 5 years) and postulated negative frequency-dependent selection as responsible mechanisms maintaining this polymorphism: the rare morph would

persistently have a selective advantage over the common one, as prey fish would accustom to being attacked more often from one side and would become more alert on that side, creating a relatively higher feeding success for the rare attacker (Hori 1993). That left-skewed and right-skewed individuals indeed feed predominantly from the right and left flanks of a prey fish, respectively, has been confirmed in experiments with one-predator:one-prey settings (e.g., Van Dooren et al. 2010; Lee et al. 2012; Takeuchi et al. 2012) as well as by stomach content analyses in wild fish (Takeuchi et al. 2016). It has further been suggested that an asymmetric mouth enables individual scale-eaters to attack from steeper rear angles thereby increasing overall feeding success as prey species have a lower probability of perceiving and avoiding the attacker (Takeuchi et al. 2012). Using simulated trophic-level food webs Nakajima et al. (2004) had already shown an evolutionary advantage for dimorphic populations of scale eaters, causing the persistence and fluctuation of this dimorphism. However, no empirical data exists to date which attributes a greater foraging success to individual scale-eaters living in dimorphic populations with respect to mouth morphology (i.e., left- and right-skewed fish together) as compared to scale-eaters in monomorphic populations (i.e., either left-skewed or right-skewed)—a main prediction if frequency-dependent selection is responsible for maintaining the polymorphism.

In this study, we report a field-based enclosure experiment with the scale-eating cichlid fish *P. microlepis* under semi-natural conditions and with interacting communities in Lake Tanganyika. We used underwater cages stocked with *P. microlepis* of differential mouth orientation as well as natural prey fish in order to (i) confirm the asymmetrical attack strategies of left-skewed and right-skewed scale-eaters under semi-natural conditions; and (ii) test the hypothesis that dimorphic scale-eater populations have an overall higher feeding success and, hence, how individual fitness is maximised by frequency-dependent selection, thereby resulting in dimorphic populations. In addition, we assessed the potential influence of habitat structures (rocky vs sandy) on the attack strategies as well as on the overall feeding success, as the more structured rocky habitat is expected to provide ample opportunity for prey fishes to hide from scale-eaters.

Materials and Methods

EXPERIMENTAL SETUP

All experiments were carried out at Kalambo Lodge (S 8° 37' 25.99" E 31° 12' 2.86") on the southern shore of Lake Tanganyika in northern Zambia during two field seasons in September 2012 and 2013 under study permit nr. 001994 (Republic of Zambia). We used the scale-eating cichlid fish *Perissodus microlepis* as predator and the common algae grazing cichlid species *Interchromis loocki* and *Tropheus moorii* as prey (Fig. 1B). Ex-

perimental fishes were caught by the authors and by local fishermen using mono-filamentous gillnets with a mesh size of 6 mm; fishes were carefully chased into the nets on snorkelling or on SCUBA and immediately removed from the nets to reduce the risk of damage to the scale cover. Prior to the experiments, fishes were kept species-wise in concrete ponds (1 × 1 × 1 m) for several days to allow them to settle and to ensure that the scale-eaters had emptied their intestines before being utilized in the experiments. *Perissodus microlepis* individuals were scored by eye and separated according to mouth orientation into two groups, those with a mouth opening to the left (left-skewed) and those with a mouth opening to the right (right-skewed). Scoring was carried out independently by three examiners (AI, AT, and WS), and fish were only used for the experiment if laterality was clearly visible and all three examiners agreed upon mouth orientation. We note that the mouth-opening angle has been suggested to be somewhat continuously distributed (see e.g., Kusche et al. 2012; Lee et al. 2015; but see Takeuchi et al. 2016) and that laterality increases with body size (Takeuchi et al. 2016). However, under field-conditions as in our study and in a situation where handling time and damage of specimens needed to be minimized, a more in-depth examination of mouth-opening angle by for example staining (Lee et al. 2015; Takeuchi et al. 2016) was not possible, and we could only use adult individuals for our experiments.

The experimental setup of this study (Fig. 1C and D) consisted of six equally sized underwater cages (2 × 2 × 2 m) made of a hollow steel frame covered by a sturdy net with 6 mm mesh size. The cages were open to the bottom to allow for the interaction of the experimental fishes with the natural substrate. The cages were installed around 30 m off shore in a water depth of 6 to 9 m. Three cages were placed on a homogeneous sandy ground, while the other three were equipped with natural rocks providing potential hiding places for prey and predator fish.

In an initial round of experiments, we carried out two trial runs to get familiar with the experimental procedure. During these trials, the condition of the experimental fishes was inspected regularly to assess attack rates of the scale-eaters. From this data, we defined the most suitable density of predator and prey fishes as well as the optimal duration of the experiment to avoid an effect of oversaturation.

For the actual experiment, consisting of three consecutive rounds in which all six cages were used, we stocked each cage with 20 prey specimens (10 *I. loocki* and 10 *T. moorii*) and 14 predators (*P. microlepis*). Within each habitat type (rocky vs sandy bottom), one cage was stocked with exclusively left-skewed *P. microlepis* (L), one with solely right-skewed individuals (R), and one with a dimorphic population (seven left-skewed and seven right-skewed individuals; M) (Fig. 1C). In doing so, we created two types of experimental populations with respect to mouth morphology of the scale-eaters: monomorphic experimental populations (L or R)

and dimorphic experimental populations (M). The assignment of these populations to individual cages was altered in rotation to avoid cage position effects. Within each experimental round, prey fish and predators were distributed according to body size among the six cages to secure a homogenous size distribution. Cages were immediately sealed upon stocking with predator and prey fish. Each experimental round lasted for three days, after which all fishes were recaptured using SCUBA and 6 mm mesh sized gillnets. Fishes were immediately euthanized with an overdose of clove-oil, and permanently stored in 96% Ethanol for transportation and long-term storage.

DATA ASSESSMENT

In a first step, we examined whether or not the attack strategy of the scale-eaters correlates with mouth asymmetry (i.e., we tested whether left-skewed scale-eaters feed more from the right body side of prey fish and right-skewed fish feed more from the left side, whereas mixed scale-eater populations were expected to feed more or less equally from both body sides of prey fish). To this end, we inspected all prey fish for missing scales on each body side in the laboratory using Leica S6E binoculars with LeicaL2 light sources. The number of missing scales was determined by two examiners (A.I. and A.T.), and the average of the two counts was taken for further transformation in order to minimize count errors. On very few prey fish, larger parts of the scale cover were missing, which could be because of predation or due to damage caused from recapturing the fish inside the cages. We thus excluded the data of the respective area on both sides of the prey fish to avoid introducing a possible bias.

In a second step, we quantified the feeding success of *P. microlepis* in relation to the different experimental conditions (i.e., we compared feeding success of the scale-eaters between the mono- and dimorphic experimental populations). To this end, we dissected the ethanol-preserved scale-eaters and inspected their intestinal tracts. We first determined whether a predator fish was able to feed at all (“feeding event”; scales present in the stomach or gut), and, in cases where predators had eaten, counted the amount of scales in the intestinal tract (“scale count”). Since very little is known about the mode as well as the rate of digestion of scales in *P. microlepis*, and since digested scales form a homogenous mass more downstream in the gut, we only counted intact or moderately digested scales from recent feeding events, which were still recognizable as discrete entities. Scale-counts could only be performed once and by one examiner (A.I.), since the specimens and their intestines were damaged during dissection.

STATISTICAL ANALYSES

All statistical analyses were done using the statistical software R (R 3.4.4; R Core Team 2016). To test for a putative correlation

between attack strategy and mouth morphology (left-skewed vs right-skewed vs dimorphic experimental populations), we categorized the absolute number of missing scales on both sides of each prey fish. In a second step, to be able to control for the varying numbers of missing scales between specimens, we categorized each prey fish into the attack strategies 0 and 1 as follows: If more scales were missing on the left flank of the prey fish than on its right side, we coded the attack strategy as 1 (i.e., the predators’ strategy was to preferentially attack the left flank of that prey fish), whereas if fewer scales were missing on the left than on the right flank, we coded it as 0 (i.e., the predators’ strategy was to preferentially attack the right flank). Data had to be categorized in this way since the distribution of counts turned out to be random so that it was not possible to transform the data. The attack strategy categories were used as response variable, together with the fixed effects mouth morph (left-skewed vs right-skewed vs dimorphic experimental populations) and habitat (rocky vs sandy), in a generalized linear-mixed model (GLMM) with a logistic link function in the R package LME4 (Bates et al. 2014) (see Table S1A). The factor “cage” was included as a random effect to account for within cage dependence of the data. We then calculated the modeled proportion of prey with more scales missing on the left side of the body when kept in the cages with either only left-skewed, only right-skewed or both together, using the probability-logit-inverse function `PLGIS`.

To analyze the feeding success of *P. microlepis* with respect to the composition of the experimental population (mono- vs dimorphic), we applied a hurdle model with the package GLMMADMB (Fournier et al. 2012; Skaug et al. 2013) (Table S1B, C, D). This model separates the data into two sets to disentangle (i) if the experimental populations showed, in general, different proportions of feeding events, and (ii) if the number of scales in the intestinal tract (“scale count”) differed among the ones with scales present in their stomach. For the first part of the hurdle procedure describing the probability for feeding events, we fitted a model to the binary part of the data, which means that all zeroes (no scales in stomach) were coded as 0 and all nonzeros (one or more scales in stomach) were coded as 1. This was done to be able to deal with zero-inflated data. We then tested, in a GLMM with logistic link function, if feeding events correlate with the experimental populations setup (mono- vs dimorphic) as a fixed effect and the factor “cage” as a random effect (Table S1B). Due to the fact that neither standard length (SL) nor habitat (rocky/sandy) improved the model significantly when included (ANOVA model comparison; $\chi^2_{\text{with SL}} = 0.174$, $p_{\text{with SL}} = 0.6766$; $\chi^2_{\text{with habitat}} = 0.012$, $p_{\text{with habitat}} = 0.9128$), these parameters were not included as additional fixed effects.

In the second part of the hurdle procedure, to compare the intestinal scale count of *P. microlepis* among the experimental populations setups (mono- vs dimorphic), a truncated negative

binomial distribution (NB1) was fitted to the nonzero outcomes of the counted intestinal scales. Additionally to the experimental populations, SL and habitat were included as fixed effects. The factor “cage” was again included as a random effect (Table S1C). The model was also repeated with the logarithmic prey:predator ratio as an offset (Table S1D) after checking for a correlation between prey:predator ratio and experimental population setups (mono- vs dimorphic). This correlation was performed with a GLMM with a logistic link function in LME4 (Bates et al. 2014), using the additional fixed effect “habitat” and the random effect “cage” (Table S1E).

Results

Overall, more than two-thirds of the experimental fish were recovered at the end of the 3-day trials. Of the initially stocked 252 specimens of *P. microlepis*, 162 were recaptured at the end of the trials; of the 360 stocked prey individuals, 260 were recaptured (*T. moorii*: 118 of 180; *I. loocki*: 142 of 180; for cage-specific sample sizes see Table S2). In addition, six nonstocked individuals were found, which were also included in further analysis since they served as prey as well. Despite the reduction in sample size, the size distribution was stable throughout the cages (mean SL \pm SD; *P. microlepis* = 78.9 ± 9.0 ; prey = 74.9 ± 12.2 ; for cage-specific SL distribution see Table S2).

All 266 recaptured prey individuals featured missing scales. In most cases, missing scales were detected on both sides of the preys' body; only 10 individuals showed missing scales exclusively on one body side. The number of missing scales was highly variable between prey specimens, ranging from 1 to 109 per specimen (mean number of missing scales \pm SD = 17.83 ± 15.67 ; for cage- and experimental population-specific information see Table S2). Overall, fewer scales were missing on the prey fish in the monomorphic experimental populations (L/R; 16.59 ± 15.09) compared to the dimorphic experimental populations (M; 22.20 ± 16.98). The proportion of prey with more scales missing on the left than on the right body side and vice versa were significantly influenced by mouth orientation of the predator in the monomorphic cages (GLMM; $n = 266$, $z = 4.227$, $P < 0.0001$; Fig. 2A), correlating with the attack strategies of *P. microlepis*, with left-skewed fish attacking from the right side whereas right-skewed ones attacked from the left side in the majority of cases. The prey from the dimorphic experimental populations showed an intermediate proportion of missing scales between the left- and right-skewed ones. That prey fish from the dimorphic experimental populations did not show an equal proportion of missing scales from both flanks of their body but had more scales missing on their left side is probably best explained by the higher recovery rate of right-skewed scale-eaters from the dimorphic cages (27 right- vs 24 left-skewed specimens;

Table S2). Contrarily to mouth morph, no effect of habitat on attack strategy was found (GLMM; $n = 266$, $z = 1.369$, $P = 0.17$).

In the second part, we tested whether *P. microlepis* of dimorphic experimental populations were more successful than monomorphic ones with regard to feeding events and the number of ingested scales. The dissection of the 162 *P. microlepis* intestines revealed that 106 individuals (65.4%) contained scales and therefore had been able to succeed at a recent feeding event (monomorphic experimental populations: 66 of 111 individuals, 59.5%; dimorphic experimental populations: 40 of 51 individuals, 78.4%). *Perissodus microlepis* therefore had a higher probability for feeding events if they were kept in cages with dimorphic experimental populations than the ones in the cages with only monomorphic experimental populations (GLMM; $n = 162$, $z = -2.32$, $P = 0.0204$; Fig. 2B).

Among the successfully feeding *P. microlepis*, we recovered between 1 and 44 scales per intestinal tract (mean intestinal scales \pm SD, range; monomorphic experimental populations = 7.5 ± 8.1 , 1–44; dimorphic experimental populations = 7.0 ± 6.8 , 1–31; for details on intestinal scale count information per cage see Table S2). The intestinal scale count was only significantly influenced by SL, but not by experimental population setup nor by habitat (GLMM; $n = 106$, $z_{\text{experimental population setup}} = 0.32$, $P_{\text{experimental population setup}} = 0.7470$; $z_{\text{SL}} = -2.13$, $P_{\text{SL}} = 0.033$; $z_{\text{habitat}} = -1.36$, $P_{\text{habitat}} = 0.1730$). These results must be taken with caution, though, as “scale count” could be influenced by the variable prey:predator ratio observed between cages (Table S2). These differences in the ratio between prey and predator fishes arose through varying sample sizes per cage due to unequal loss of experimental individuals, which is difficult to avoid in a semi-natural setting such as ours. Main reasons for losses in our experiment might be complications with recompression (note that fishes had to be brought to a depth of 6 to 9 m) as well as territorial fights within cages. When correcting for variable prey:predator ratios, we found that successfully feeding scale-eaters in the dimorphic experimental populations do have a higher feeding rate compared to the ones in monomorphic experimental populations (GLMM; $n = 106$, $z = -3.17$, $P = 0.0015$). Again, feeding rate was significantly influenced by SL here, but not by habitat (GLMM; $n = 106$; $z_{\text{SL}} = -2.81$, $P_{\text{SL}} = 0.0049$; $z_{\text{habitat}} = -1.75$, $P_{\text{habitat}} = 0.0801$). We note, however, that correcting for prey:predator ratio might itself introduce a bias by acting as a confounding factor. The average prey:predator ratio was – probably coincidentally – lower in dimorphic experimental populations than in monomorphic ones, which was not explainable by habitat (GLMM; $n = 106$, $z_{\text{experimental population setup}} = 3.131$, $P_{\text{experimental population setup}} = 0.0017$; $z_{\text{habitat}} = 0.059$, $P_{\text{habitat}} = 0.9530$). Therefore, the correlations of prey:predator ratio with the response variables intestinal scale count and the fixed effect experimental population setup cannot be disentangled. We would also like to note that the

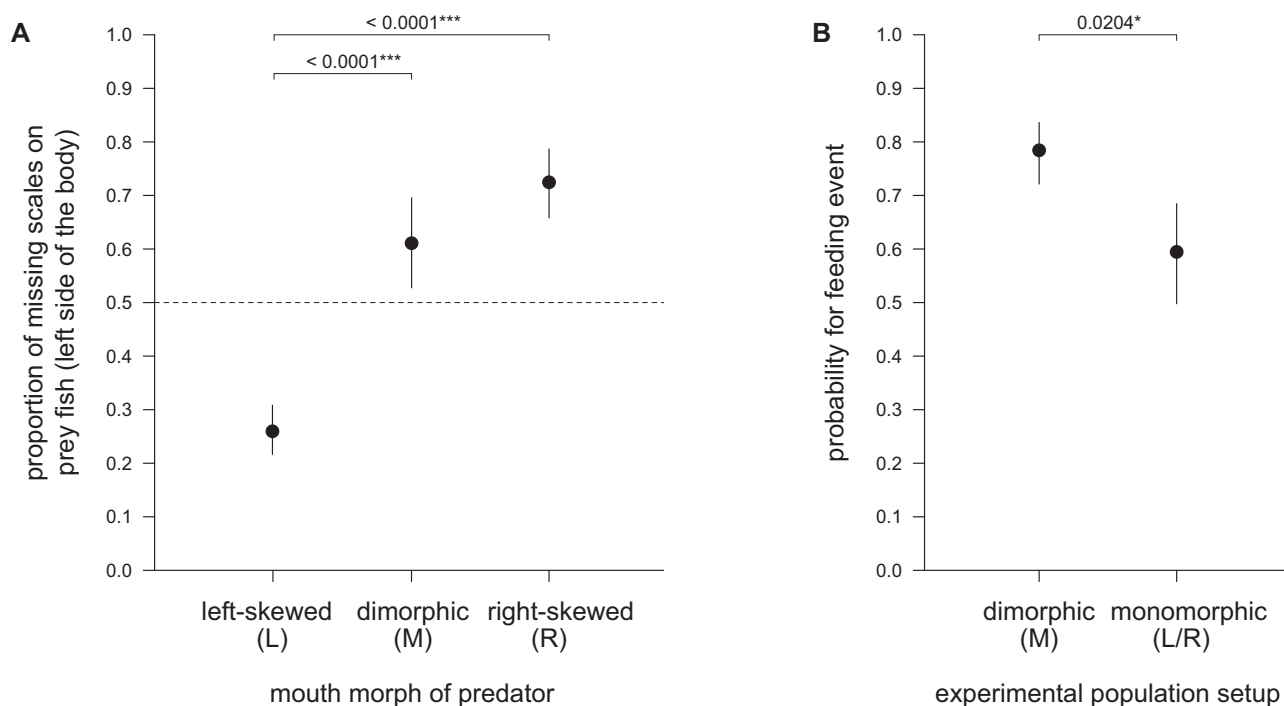


Figure 2. Attack strategies and feeding events in experimental scale-eater populations. (A) Attack strategy as the proportions of missing scales on the prey species' left body side for the separate mouth morphs as well as the dimorphic experimental populations. (B) Probability for a feeding event in dimorphic or monomorphic experimental population setups. *P*-values are shown for significant tests only.

models with and without offset (i.e., correction for prey:predator ratio) resulted in nearly identical AIC values and should thus both be taken into consideration.

Discussion

Trophic polymorphisms—that is, the persistence of two or more discrete trophic-related phenotypes within a single species—occur in many vertebrate species and are often manifested in differences in structural features such as jaws, teeth, or beaks, influencing feeding performance (Skulason and Smith 1995). Several species of cichlid fishes, for example, show a trophic polymorphism in the size, shape, and dentition of their pharyngeal jaw bones—that is, a trophic structure in their pharynx used to process food—and it has been shown that different pharyngeal jaw morphs perform differently with respect to hard vs soft diet (Meyer 1989; Hulseley et al. 2005).

In this study, we report a field-based enclosure experiment in a semi-natural environment to assess attack strategies and feeding success of another type of trophic polymorphism in cichlids: the orientation of mouth opening in the scale-eating cichlid *Perissodus microlepis* in Lake Tanganyika in East Africa. In a first step, by examining the missing scales on prey fishes exposed to scale-eaters in underwater cages, we confirm previous findings on the attack strategy of *P. microlepis* (Takeuchi et al. 2012; Lee et al. 2012; Takeuchi et al. 2016). We show that under semi-natural circumstances and with community interactions, the two mouth

morphs show a feeding preference on the respectively most suitable flank of the prey (i.e., left-skewed fish feed preferably from the right flank of prey fishes, while right-skewed individuals attack more often the left side) (Fig. 2A). In addition, we show that in mixed (dimorphic) scale-eater experimental populations, the number of missing scales on the prey fish was near intermediate (Fig. 2A). In contrast to previous work reporting relatively few (ca. 20% in Takeuchi et al. 2012) or no (in Lee et al. 2012) attacks to the “wrong” flank of the prey, our field- and community-based experiments with monomorphic populations revealed that scale-eaters regularly feed from the “wrong” side of the prey as well; notably, only 10 out of 207 prey fish in the monomorphic populations had been attacked at only one side. The difference between previous studies and our work is most likely explained by the different experimental settings: while Takeuchi et al. (2012) and Lee et al. (2012) used one-predator:one-prey setups, we opted for a community setting with several predator and prey fishes in semi-natural conditions using underwater cages in the natural habitat of both the predators and the prey. It thus seems likely that scale-eaters depart from their optimal hunting strategy (the one uncovered in one-predator:one-prey experiments) under semi-natural or natural conditions, where fishes encounter each other in differing orientations and on multiple occasions. Alternatively, the relatively high rate of attacks to the “wrong” flank might be an indication that our setup provided the scale-eaters with more opportunities for strikes, for example due to the slightly elevated

prey density compared to natural communities and the lack of dilution by other species (Sturmbauer et al. 2008).

In a second step, by counting the scales from the intestinal tracts of *P. microlepis*, we determined the feeding success of scale-eaters in mono- vs dimorphic populations, whereby feeding success is composed of two factors, which were analyzed separately here: (i) the opportunity to feed as defined by whether or not an actual feeding event has taken place, and (ii) the number of ingested scales in the intestinal tract of a scale-eater. Importantly, the probability to feed was greater in scale-eaters living in a dimorphic experimental population than in individuals in monomorphic populations (Fig. 2B). This seems to be attributable to the fact that—in a dimorphic population—prey specimens have a lowered chance to adapt to the attack strategies of the scale-eater (as strikes occur toward both flanks) as compared to a monomorphic population (where strikes occur predominantly toward one flank with higher frequency; Fig. 2A). The intestinal scale counts provided a less clear picture as to whether dimorphic scale-eater populations have a selective advantage over monomorphic ones. Only when correcting for differing prey:predator ratios in the different cages did we find that scale-eaters had a significantly higher feeding success in the dimorphic experimental populations.

Interestingly, the habitat structure (sandy vs rocky) did not have any effect on either the attack strategy or on feeding success, which might be an effect of the limited sample size. Nevertheless, this result is somewhat surprising, given that the rocky habitat provides ample opportunity for prey fishes to hide from predators as well as for predators to ambush their prey. Interestingly, we found that smaller sized scale-eaters feature a higher feeding rate compared to larger one's, which is different to what Takeuchi et al. (2016) have found in fishes taken from the wild. One explanation might be that in our enclosures, smaller sized predators are less likely to be detected by the prey fish or might have diminished intimidation effects on them.

Taken together, our study is the first experimental demonstration that individual scale-eating cichlid fish have a significantly increased chance of striking a successful attack when living in dimorphic compared to monomorphic populations, suggesting that the higher probability for feeding—possibly resulting in a higher feeding rate—is the selective agent responsible for the evolution and maintenance of mouth dimorphism in *P. microlepis*. Our results lend further support to the hypothesis that negative frequency-dependent selection is the stabilizing force responsible for balancing the mouth dimorphism at a quasi-equal ratio in natural populations (Hori 1993). The two mouth morphs of *P. microlepis* can thus be viewed as two distinct natural groups with respect to attack strategy that, based on our results, persist within a single interbreeding species for the reason that the selective advantage of the trait in question arises primarily through its intrinsic bimodality.

AUTHOR CONTRIBUTIONS

A.I., A.T. and W.S. designed the study. A.I., A.T., B.E., and W.S. conducted the experiments in the field. A.I. and A.T. performed the scale counts. A.I., A.T. and W.S. analyzed the data. A.I., A.T., B.E., and W.S. wrote and edited the manuscript.

ACKNOWLEDGMENTS

We are grateful to the fisheries department of Zambia, Mpulungu (especially to Dr. Lawrence Makasa and Dr. Dani Syninza) for research permits and support in the field. We thank Gilbert Tembo with his family, crew, and the local fishermen at Kalambo Lodge for their ever welcoming and hospitable nature and their indispensable help. We are further thankful to Yuri Kläfiger, Fabrizia Ronco, Marie Dittmann, and Hugo Gante for their help with SCUBA in the field. We owe gratitude to Carolin Göppert and Lukas Widmer for valuable help and discussions in developing the protocol for data assessment, Tobias Roth and Fabrizia Ronco for advice in the statistical analyses, Angel M. Fitor for providing underwater photographs, and to Heinz Büscher for the x-ray images. We would also like to thank the Associate Editor, Dr. Simone Immler, as well as two anonymous reviews for their valuable comments. A.I. was funded by the University of Basel and the Freiwillige Akademische Gesellschaft Basel (FAG). A.T. was funded by the University of Basel, the Freiwillige Akademische Gesellschaft Basel (FAG), and the Swiss Zoological Society (SZS). W.S. received funding through the European Research Council (ERC) Starting Grant “INTERGENADAPT” and Consolidator Grant “CICHLID~X,” the University of Basel and the Swiss National Science Found (SNF).

DATA ARCHIVING

The doi for our data is <https://doi.org/10.5061/dryad.q7h8nv1>.

LITERATURE CITED

- Ayala, F. J., and C. A. Campbell. 1974. Frequency-dependent selection. *Annu. Rev. Ecol. Syst.* 5:115–138.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014 *lme4: linear mixed-effects models using Eigen and S4*. R package version 1.0-6. <http://CRAN.R-project.org/package=lme4>
- Boileau, N., F. Cortesi, B. Egger, M. Muschick, A. Indermaur, A. Theis, H. H. Büscher, and W. Salzburger. 2015. A complex mode of aggressive mimicry in a scale-eating cichlid fish. *Biol. Lett.* 11:20150521.
- Clarke, B., and P. O'Donald. 1964. Frequency-dependent selection. *Heredity* 19:201–206.
- Darwin, C. R. 1859. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. John Murray, London, U.K.
- Fournier, D. A., H. J. Skaug, J. Ancheta, J. Ianelli, A. Magnusson, M. N. Maunder, A. Nielsen, and J. Sibert. 2012. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optim. Method Softw.* 27:233–249.
- Friedman, M. 2008. The evolutionary origin of flatfish asymmetry. *Nature* 454:209–212.
- Fryer, G., and T. D. Iles. 1972. *The cichlid fishes of the Great Lakes of Africa: their biology and evolution*. Oliver and Boyd, Edinburgh, U.K.
- Futuyama, D. J. 2009. *Evolution*. Sinauer Associates, Sunderland, MA.
- Gigord, L. D., M. R. Macnair, and A. Smithson. 2001. Negative frequency-dependent selection maintains a dramatic flower color polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soò. *Proc. Natl. Acad. Sci. USA* 98:6253–6255.
- Govind, C. K. 1989. Asymmetry in lobster claws. *Am. Sci.* 77:468–474.
- Hori, M. 1993. Frequency-dependent natural selection in the handedness of scale-eating cichlid fish. *Science* 260:216–219.

- Hori, M., H. Ochi, and M. Kohda M. 2007. Inheritance pattern of lateral dimorphism in two Cichlids (a scale eater, *Perissodus microlepis*, and an herbivore, *Neolamprologus moorii*) in Lake Tanganyika. *Zool. Sci.* 24:486–492.
- Hori, M., and K. Watanabe. 2000. Aggressive mimicry in the intrapopulation color variation of the Tanganyikan scale-eater *Perissodus microlepis* (Cichlidae). *Environ. Biol. Fish.* 59:111–115.
- Hulsey, C. D., D. A. Hendrickson, and F. J. Garcia de Leon. 2005. Trophic morphology, feeding performance and prey use in the polymorphic fish *Herichthys minckleyi*. *Evol. Ecol. Res.* 7:1–22.
- Kobl Müller, S., B. Egger, C. Sturmbauer, and K. M. Sefc. 2007. Evolutionary history of Lake Tanganyika's scale-eating cichlid fishes. *Mol. Phylogenet. Evol.* 44:1295–1305.
- Koskella, B., and C. M. Lively. 2009. Evidence for negative frequency-dependent selection during experimental coevolution of a freshwater snail and a sterilizing trematode. *Evolution* 63:2213–2221.
- Kusche, H., H. J. Lee, and A. Meyer. 2012. Mouth asymmetry in the textbook example of scale-eating cichlid fish is not a discrete dimorphism after all. *Proc. R Soc. B* 279:4715–4723.
- Lee, H. J., S. Pittlik, J. C. Jones, W. Salzburger, M. Barluenga, and A. Meyer. 2010. Genetic support for random mating between left and right-mouth morphs in the dimorphic scale-eating cichlid fish *Perissodus microlepis* from Lake Tanganyika. *J. Fish. Biol.* 76:1940–1957.
- Lee, H. J., H. Kusche, and A. Meyer. 2012. Handed foraging behavior in scale-eating cichlid fish: its potential role in shaping morphological asymmetry. *PLoS ONE* 7:e44670.
- Lee, H. J., V. Heim, and A. Meyer. 2015. Genetic and environmental effects on the morphological asymmetry in the scale-eating cichlid fish, *Perissodus microlepis*. *Ecol. Evol.* 5:4277–4286.
- Liem, K. F., and D. J. Stewart. 1976. Evolution of the scale-eating cichlid fishes of Lake Tanganyika: a generic revision with a description of a new species. *Bull. Mus. Comp. Zool.* 147:313–350.
- Marlier, G., and N. Leleup. 1954. A curious ecological niche among the fishes of Lake Tanganyika. *Nature* 174:935–936.
- Meyer, A. 1989. Cost of morphological specialization: feeding performance of the two morphs in the trophically polymorphic cichlid fish, *Cichlasoma citrinellum*. *Oecologia* 80:431–436.
- Muschick, M., A. Indermaur, and W. Salzburger. 2012. Convergent evolution within an adaptive radiation of cichlid fishes. *Curr. Biol.* 22:2362–2368.
- Nakajima, M., H. Matsuda, and M. Hori. 2004. Persistence and fluctuation of lateral dimorphism in fishes. *Am. Nat.* 163:692–698.
- Nosil, P. 2012. *Ecological speciation*. Oxford Univ. Press, Oxford, U.K.
- Palmer, A. R. 1994. Fluctuating asymmetry analyses: a primer. Pp. 335–364 in T. A. Markow, ed. *Developmental instability: Its origins and evolutionary implications*. Kluwer Academic Publisher, Dordrecht, The Netherlands.
- . 2004. Symmetry breaking and the evolution of development. *Science* 306:828–833.
- . 2005. Antisymmetry. Pp. 359–397 in Hallgrímsson, B., and B. K. Hall, eds. *Variation: A central concept in biology*. Academic Press, New York, NY.
- . 2009. Animal asymmetry. *Curr. Biol.* 19:R473–R477.
- . 2010. Scale-eating cichlids: from hand(ed) to mouth. *J. Biol.* 9:11.
- R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing: Vienna, Austria.
- Raffini, F., C. Fruciano, P. Franchini, and A. Meyer. 2017. Towards understanding the genetic basis of mouth asymmetry in the scale-eating cichlid *Perissodus microlepis*. *Mol. Ecol.* 26:77–91.
- Schreiber, A. M. 2006. Asymmetric craniofacial remodeling and lateralized behavior in larval flatfish. *J. Exp. Biol.* 209:610–621.
- Smithson, A., and M. R. Macnair. 1997. Negative frequency-dependent selection by pollinators on artificial flowers without rewards. *Evolution* 51:715–723.
- Stewart, T., and R. C. Albertson. 2010. Evolution of a unique predatory feeding apparatus: functional anatomy, development and a genetic locus for jaw laterality in Lake Tanganyika scale-eating cichlids. *BMC Biol.* 8:8.
- Sturmbauer, C., C. Fuchs, G. Harb, E. Damm, N. Duftner, M. Maderbacher, M. Koch, and S. Kobl Müller. 2008. Abundance, distribution, and territory areas of rock-dwelling Lake Tanganyika cichlid fish species. *Hydrobiologia* 615:57–68.
- Skaug, H., D. Fournier, A. Nielsen, A. Magnusson, and B. Bolker. 2012. Generalized linear mixed models using AD Model Builder. R package version 0.7.7.
- Skulason, S., and T. B. Smith. 1995. Resource polymorphism in vertebrates. *Trends Ecol. Evol.* 10:366–370.
- Takahashi, R., T. Moriwaki, and M. Hori. 2007a. Foraging behaviour and functional morphology of two scale-eating cichlids from Lake Tanganyika. *J. Fish. Biol.* 70:1458–1469.
- Takahashi, R., K. Watanabe, M. Nishida, and M. Hori. 2007b. Evolution of feeding specialization in Tanganyikan scale-eating cichlids: a molecular phylogenetic approach. *BMC Evol. Biol.* 7:195.
- Takahashi, T., and M. Hori. 2008. Evidence of disassortative mating in a Tanganyikan cichlid fish and its role in the maintenance of intrapopulation dimorphism. *Biol. Lett.* 4:497–499.
- Takeuchi, Y., M. Hori, and Y. Oda. 2012. Lateralized kinematics of predation behavior in a Lake Tanganyika scale-eating cichlid fish. *PLoS ONE* 7:e29272.
- Takeuchi, Y., M. Hori, S. Tada, and Y. Oda. 2016. Acquisition of lateralized predation behaviour associated with development of mouth asymmetry in a Lake Tanganyika scale-eating cichlid fish. *PLoS ONE* 11:e0147476.
- Takeuchi, Y., and Y. Oda. 2017. Lateralized scale-eating behaviour of cichlid is acquired by learning to use the naturally stronger side. *Sci. Rep.* 7:8957.
- Van Dooren, T. J. M., H. A. Van Goor, and M. Van Putten. 2010. Handedness and asymmetry in scale-eating cichlids: antisymmetries of different strength. *Evolution* 64:2159–2165.
- Van Valen, L. 1962. A study of fluctuating asymmetry. *Evolution* 16:125–142.

Associate Editor: S. Immler
Handling Editor: P. Tiffin

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Table 1. Models used to test for a correlation of attack strategy and experimental population (L, M, and R) (A), probability for a feeding event and experimental population setup (mono- vs dimorphic) (B), scale count and experimental population setup (mono- versus dimorphic) without a correction for prey:predator ratio (C) and with a correction for prey:predator ratio (D) as well as for a correlation of prey:predator ratio and experimental population setup (mono- vs dimorphic) (E).

Supplementary Table 2. Cage- and experimental population-specific information on sample sizes for predator and prey specimen, prey:predator ratios, left-to-right-skewed-ratios, standard lengths and scale information (missing scales on the prey's body side as well as intestinal scale counts for *P. microlepis*).