

Relaxed Feeding Constraints Facilitate the Evolution of Mouthbrooding in Neotropical Cichlids

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ABSTRACT: Multifunctionality is often framed as a core constraint of evolution, yet many evolutionary transitions involve traits taking on additional functions. Mouthbrooding, a form of parental care where offspring develop inside a parent's mouth, increases multifunctionality by adding a major function (reproduction) to a structure already serving other vital functions (feeding and respiration). Despite increasing multifunctionality, mouthbrooding has evolved repeatedly from other forms of parental care in at least seven fish families. We hypothesized that mouthbrooding is more likely to evolve in lineages with feeding adaptations that are already advantageous for mouthbrooding. We tested this hypothesis in Neotropical cichlids, where mouthbrooding has evolved four or five times, largely within winnowing clades, providing several pairwise comparisons between substrate-brooding and mouthbrooding sister taxa. We found that the mouthbrooding transition rate was 15 times higher in winnowing than in nonwinnowing clades and that mouthbrooders and winnowers overlapped substantially in their buccal cavity morphologies, which is where offspring are incubated. Species that exhibit one or both of these behaviors had larger, more curved buccal cavities, while species that exhibit neither behavior had narrow, cylindrical buccal cavities. Given the results we present here, we propose a new conceptual model for the evolution of mouthbrooding, integrating the roles of multifunctional morphology and the environment. We suggest that functional transitions like mouthbrooding offer a different perspective on multifunctionality: increasing constraints in one trait may release them for another, generating new evolutionary opportunities.

Keywords: life history, mouthbrooding, parental care, feeding, multifunctionality, cichlids.

Introduction

Throughout an organism's lifetime, most structures will serve more than one function. This multifunctionality is often framed as a core constraint of phenotypic evolution: the more functions a structure directly serves, the more the evolution of that structure is constrained by the increasingly narrow range of phenotypes that will sufficiently serve all of those functions (Corn et al. 2021; Gilbert et al. 2021). Most of the functions an animal must perform—feeding, respiration, locomotion, and reproduction—do not change even if their particular mode (e.g., swimming or flying) does. Instead, many evolutionary transitions involve a reshuffling of which structures are primarily responsible for which functions. Constraints at this scale, therefore, are displaced from one structure to another more often than they are eliminated. In tetrapod evolution, for example, respiration transitioned from the head (buccal pumping) to the trunk (costal ventilation), relieving the head of this function by shifting it to the trunk, which is already serving in locomotion (Carrier 1987; Janis and Keller 2001; Brainerd and Owerkowicz 2006; Perry and Carrier 2006; Dial et al. 2015).

A transition that decreases multifunctionality (presumably easing constraint) has clear advantages for the unburdened structure, but it increases multifunctionality for the newly burdened structure. Even if increasing multifunctionality can potentially contribute to evolvability (Pigliucci and Preston 2004; Parsons et al. 2012; Thiagavel et al. 2018; Conith et al. 2019), this is unlikely to provide an immediate selective advantage. Why, then, would such transitions ever occur under natural selection? One compelling hypothesis is that they are more likely when the structure taking on an additional function is already well suited to that function before the transition takes place

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(e.g., costal ventilation may have co-opted intercostal muscles that evolved first for locomotion; Gould and Vrba 1982; Cieri et al. 2018). But most major evolutionary transitions are singular by nature, making it difficult to attribute them to one causal explanation over another (Felsenstein 1985; Dial et al. 2015; Maddison and FitzJohn 2015).

Mouthbrooding, a common form of parental care in fishes in which the offspring are incubated in the parent's mouth, could be a helpful trait in understanding how multifunctionality increases. It is certainly a puzzling one: by housing their offspring in their mouths, mouthbrooders use a feeding structure as a nursery, tangling together selective pressures that were previously as uncoupled as any two functions can be on the same organism (Okuda et al. 2002; Östlund-Nilsson and Nilsson 2004; Hoey et al. 2012; Verheyen et al. 2012; Van Wassenbergh et al. 2015). The behavior is hypothesized to evolve in response to particular environmental factors of offspring mortality, particularly predation, hypoxia, and nest site availability, which is supported by the observation that most mouthbrooding species (across at least seven fish families) occur in spatially complex warm-water environments with high predation rates (Shaw and Aronson 1954; Blumer 1982; Mrowka 1984).

But despite its seemingly widespread phylogenetic distribution, mouthbrooding is never the most common parental care strategy among fishes in a given environment, even among lineages that provide other forms of parental care (e.g., equilibrium strategists sensu Winemiller and Rose 1992). Fishes exhibit a notoriously wide range of parental care strategies, many of which (like live-bearing or external brood carrying) should confer the same advantages as mouthbrooding without increasing multifunctionality among adaptive structures (Berra and Humphrey 2002; Pietsch et al. 2009). So why has mouthbrooding evolved repeatedly in fishes that already exhibit other forms of parental care, and why has it evolved in some lineages but not others?

Here, we hypothesize that mouthbrooding may be more likely to evolve in lineages with feeding modes that were already well suited for mouthbrooding. Precisely what makes a feeding mode well suited for mouthbrooding probably varies, and feeding traits that may be co-opted in parental care range from behavioral neural circuitry (Fischer and O'Connell 2017) and motor control patterns (Barkan and Zornik 2019) to cranial and branchial morphology (Hoey et al. 2012; O'Connor et al. 2012; Weller et al. 2017). We can make some generalizations based on the physiological requirements of incubating eggs: at a minimum, the upper limit on brood size is set by the upper limit on mouth volume, as opposed to batch fecundity or defensible nest size (fig. 1). Beyond that, any aspect of the oral cavity that contributes to increased water flow over the eggs (Shaw and Aronson 1954) should also be advantageous.

We chose to test this hypothesis in Neotropical cichlids. Mouthbrooding has evolved several times within the clade (four to seven, per Goodwin et al. 1998), and the splits between mouthbrooders and substrate brooders often occur within genera that otherwise have highly conserved phenotypes (López-Fernández et al. 2014). The relatively few differences between mouthbrooding and substrate-brooding sister taxa, usually including their shared feeding strategies and morphologies, provide us with much better opportunities to disentangle trait changes associated with mouthbrooding (López-Fernández et al. 2012, 2013). Unlike most mouthbrooding taxa (including African cichlids), most mouthbrooding Neotropical cichlids exhibit biparental care and little sexual dimorphism (Stawikowski and Werner 2004), minimizing the effects of what could be major confounding variables. Last, many Neotropical cichlids—including most of the mouthbrooding species—are winnowers that feed by sifting mouthfuls of substrate for invertebrates or edible detritus (Weller et al. 2017). Winnowing and mouthbrooding, at least superficially, are strikingly similar behaviors in

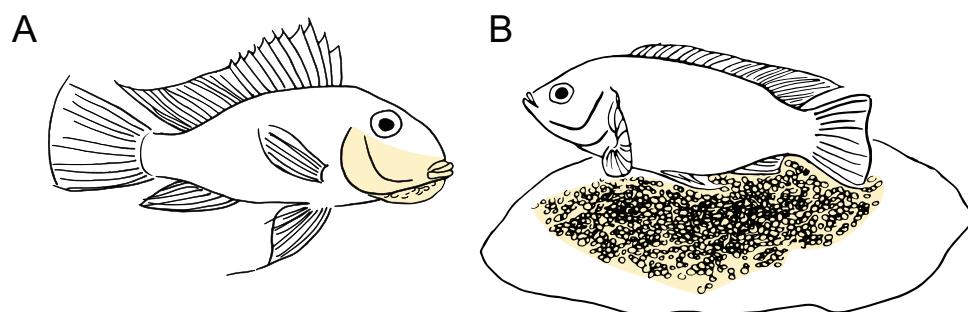


Figure 1: Mouthbrooding and substrate-brooding parental care behaviors in cichlids. Only egg care behaviors are shown, but both forms of parental care can also extend to care of the fry. Locations of the eggs are highlighted in yellow. *A*, Mouthbrooding. Note the visible bulge in the buccal cavity (the "brood pouch") where the hyoid is depressed to make space for the eggs or fry. *B*, Substrate brooding. In addition to guarding the area where the eggs are laid, parents will frequently pick at the eggs with their mouths or fan the eggs with their pectoral fins.

Neotropical cichlids, involving intraoral manipulation of small objects (eggs or substrate) via repeated cycles of premaxillary protrusion, hyoid depression, and opercular flaring (in contrast with most African lake mouthbrooders, which pack the expanded buccal cavity with eggs and maintain a noticeable bulge at the hyoid; Van Wassenbergh et al. 2016; Weller et al. 2017). This makes Neotropical cichlids an excellent candidate clade to test the hypothesis that mouthbrooding co-opts substrate-sifting morphology.

We focus specifically on the morphology of the buccal cavity because this is where the offspring are housed in mouthbrooding and where substrate is processed for winnowing, meaning that it should be directly impacted by both functions. If feeding mode does influence the evolution of mouthbrooding, then we should see that (1) mouthbrooding evolves more frequently from winnowing than from other feeding modes; (2) winnowing is morphologically distinct from other feeding modes (López-Fernández et al. 2012, 2014), including increased buccal cavity volumes; and (3) mouthbrooding is associated with larger morphological change if it evolves in a lineage with a conflicting feeding mode.

Material and Methods

All data and code used in this study are stored in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.4f4qrjdc>; Weller et al. 2021).

Transitions in Parental Care and Feeding Mode

Parental Care and Feeding Mode Classifications. We used a 559-species Neotropical cichlid phylogeny trimmed from the cichlid phylogeny presented in McGee et al. (2020), which includes more species but is otherwise congruent with other Neotropical cichlid phylogenies (López-Fernández et al. 2013; Ilves et al. 2018). We classified every species in this tree for which data were available as either a winnower or a nonwinnower and as either a mouthbrooder or a substrate brooder, so that every species was categorized as one of four possible states: (1) mouthbrooding and winnowing, (2) mouthbrooding and nonwinnowing, (3) substrate brooding and winnowing, or (4) substrate brooding and nonwinnowing. Parental care data were gathered from Stawikowski and Werner (2004), Goodwin et al. (1998), and López-Fernández et al. (2012), and winnowing data were gathered from López-Fernández et al. (2014), Weller et al. (2017), and López-Fernández et al. (2012). Because winnowing and mouthbrooding encompass a range of behaviors, we classified species inclusively for each category: a species was classified in a behavioral category if individuals exhibit the behavior to any degree that it could impact their morphology. The variations of each behavior (e.g., mouthbrood-

ing eggs or mouthbrooding larvae) probably pose slightly different constraints, but because we lacked sufficient phylogenetic and behavioral data to differentiate these fine-scaled differences, here we focus on their aggregate effects. Any species in which at least one sex orally incubates the offspring during at least one developmental period (eggs, larvae, or juveniles) was classified as a mouthbrooder. Similarly, any species that feeds by orally sifting substrate for invertebrates or edible detritus was classified as a winnower, even if it also feeds using other strategies. If we could not reliably determine whether a species is a mouthbrooder—for example, if it was most commonly reported as substrate brooding and only rarely reported as mouthbrooding in aquaria (Staeck and Schindler 2015) or if mouthbrooding and substrate-brooding behaviors seemed highly variable even in captivity (Breeze 2007; Römer et al. 2017)—it was conservatively classified as a substrate brooder (four species total). We also ran our comparative analyses below on versions of the data set with these species (1) classified as mouthbrooders and (2) excluded from the data set altogether to assess the sensitivity of our results to these ambiguous cases.

Discrete Trait Evolution. We tested for correlated evolution of mouthbrooding and winnowing as binary characters using the method described by Pagel (1994), implemented using the fitPagel function in the phytools package in R (Revell 2012). To estimate the number of times that mouthbrooding has evolved from winnowing compared with nonwinnowing clades, we combined the two traits into a four-state discrete character (see the previous section) and ran 10,000 stochastic character maps under three different transition rate models (equal rates, symmetrical rates, and all-rates-different models) to simulate transitions between these states along the phylogeny. An equal rates model assumes that all transitions, in any direction, occur at the same rate (one parameter); a symmetrical rates model assumes that transitions between states occur at the same rate but that those rates differ between pairs of states (e.g., $A \rightarrow B = B \rightarrow A$ but $A \rightarrow B \neq A \rightarrow C$); and an all-rates-different model fits a different rate parameter for each kind of transition. We also fit Mk models of discrete character evolution (fitMk in phytools) under the same three models (Lewis 2001). These models were compared using their Akaike information criterion (AIC) weights, and we calculated the weighted average of the three sets of stochastic character maps using these weights.

Buccal Cavity Morphology

Data Collection and Landmarking. For morphological analysis, we sampled 41 species across 20 genera representing the major radiations of Neotropical cichlids, mostly

from the University of Michigan Museum of Zoology and the Field Museum of Natural History. Minimally, we sampled to the resolution of the node representing the most recent common ancestor between mouthbrooding and non-mouthbrooding taxa for each clade of mouthbrooding species, so that we sampled the immediate substrate-brooding sister taxon for each mouthbrooding taxon. Where possible, we sampled three to five individuals per species, except in cases where fewer than three individuals were available in collections ($n = 17$); see the supplemental PDF, available online, for accession numbers and sample sizes for each species. We selected for analysis only specimens that were fixed in closed-mouth, neutral positions (i.e., no opercular flaring or hyoid depression). Because most mouthbrooding Neotropical cichlids are biparental and exhibit little to no sexual dimorphism, we did not distinguish between males and females in our sampling. The exception is *Gymnogeophagus*, which exhibits maternal-only care and extreme sexual dimorphism; we still sampled males and females from mouthbrooding *Gymnogeophagus* to be consistent with the rest of the study.

Each specimen was dissected using a modified version of the procedure described by Ridewood (1904) to remove the right suspensorium and oral jaw elements, exposing the buccal cavity and branchial arches (fig. 2). First, we made a midline cut on an anterior-posterior axis separating the premaxillary bones and continued this cut by bisecting the parasphenoid along the midline. We separated the circumorbital bones from around the orbit before cutting the levator operculi. Ventrally, we separated the basihyal from its articulation with the urohyal and basibranchials, then cut the branchiostegal membrane from the urohyal through

the mandibular symphysis. We were then able to remove the entire right cheek intact, including the opercular series, suspensorium, adductor mandibulae, nasal and circumorbital bones, and oral jaws.

We photographed specimens before and after dissection on a Leica or Zeiss stereomicroscope to capture both external and buccal cranial morphologies. We based our external landmarks on those in Weller et al. (2017), including curves for the rostrum, eye, lower jaw, operculum, and adductor mandibulae complex (fig. 2A). For the buccal cavity, we chose landmarks describing structures that make up the borders of the cavity, including the vomer, parasphenoid, basibranchial series, hyoid, and lower jaw. A full list of fixed and sliding landmarks is given in the supplemental PDF.

Photographs were scaled and landmarked using StereoMorph (Olsen and Westneat 2015), then imported into geomorph using the readland.shapes function, which converts StereoMorph curves into sliding semilandmarks (Adams and Otárola-Castillo 2013).

Comparative Analysis of Buccal Cavity Area and Shape. For each dissected specimen, we measured the area of the buccal cavity in sagittal section (in square millimeters). To test whether taking the surface area of the sagittal section of the buccal cavity was an accurate proxy for its 3D volume, we measured the 3D buccal cavity volumes and 2D sagittal section areas of 20 cichlid microcomputed tomography scans taken at the University of Michigan (fig. S1; figs. S1–S5 are available online). Scans were segmented using the open-source software 3D Slicer (Fedorov et al. 2012; Kikinis et al. 2014). Surface area and volume were tightly correlated ($R^2 = 0.96$, $P = 2.67 \times 10^{-14}$), with an average

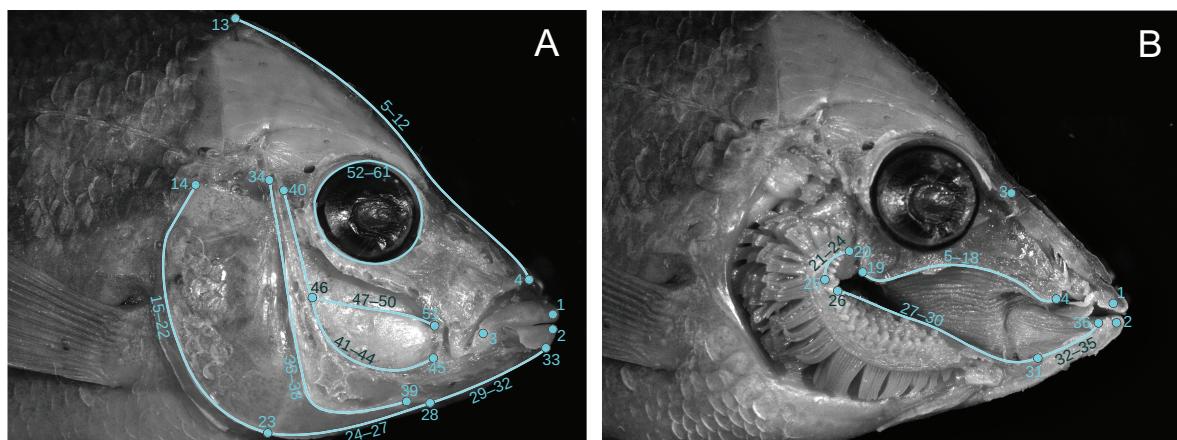


Figure 2: Landmarking scheme for morphological analysis based on dissections. Lines indicate curves for sliding landmarks; points indicate fixed landmarks. *A*, External landmark scheme. *B*, Buccal cavity landmark scheme. See the supplemental PDF for a list of fixed landmarks and curves.

error in predicted volume of 0.41% (fig. S2), establishing sagittal section area as a highly accurate proxy for buccal cavity volume (see sec. S1 of the supplemental PDF for details).

To correct for the effect of body size, we took the residuals of a log-log model regressing individual specimen buccal cavity area on squared standard length before pooling observations by species. These residuals were then averaged for each species. We tested for the effect of parental care and feeding mode on buccal cavity area using a phylogenetic generalized least squares (PGLS) model, implemented via the procD.pgl function in geomorph, with squared standard length as a covariate to account for body size (Adams and Collyer 2018). We also corrected for body size using the phyl.resid function in phytools (regressing area on squared standard length and then fitting those residuals as a function of behavioral categories with procD.pgl) as an alternate method of body size correction.

For comparative geometric morphometrics, we used geomorph to perform Procrustes superimposition of both the head and the buccal cavity landmark sets. As with buccal cavity area, we used the procD.pgl function to fit buccal cavity shape as a function of feeding and parental care modes to test for the effect of mouthbrooding and winnowing on buccal cavity shape. To visualize the distribution of buccal cavity shapes, we also performed a principal components analysis and used the method described by Olsen (2017) to produce predicted (back-transformed) shapes for the first two principal components (PCs).

Results

Mouthbrooding Transition Rates among Winnowers and Nonwinnowers

Using Pagel's (1994) method for testing the correlated evolution of discrete characters, we found the highest support (72% by AIC weight) for a model where parental care type depends on feeding mode but feeding mode does not depend on parental care type. The next best fit, mutual dependence of parental care type and feeding mode, had rea-

sonable support ($\Delta\text{AIC} = +2.2$ compared with the best model, 24% AIC weight), followed by the mutual independence model ($\Delta\text{AIC} = +6.0$, 3% AIC weight) and feeding mode depending on parental care type ($\Delta\text{AIC} = +8.4$, 1% AIC weight).

Of the three different rate assumption M_k models we fit for the four discrete behavioral categories, the symmetrical rates model was best supported (86.9% AIC weight), followed by the all-rates-different model (12.8%); the equal rates model had very little support (0.3% AIC weight). We used these AIC weights to calculate the weighted average of the transition rate matrices (table 1). The mouthbrooding transition rate was 15 times higher in winnowers than in nonwinnowers and 2.5 times higher than the transition rate to or from winnowing itself.

For the 10,000 simulated stochastic character maps for each rate model, we randomly sampled a proportion equal to the AIC weight for each model (8,690 symmetrical rate maps, 1,280 all-rates-different maps, and three equal rates maps) for a combined total of 10,000 stochastic character maps. Across these simulations, mouthbrooding evolved from substrate brooding and winnowing a median value of three times, compared with only once from substrate brooding and nonwinnowing (fig. 3). The high probability density (HPD) interval for winnowing to winnowing and mouthbrooding was also considerably wider than it was for any other transition (zero to five times). Winnowing itself was gained a median of seven times (HPD interval of six to nine), more than double the number of times that mouthbrooding was gained. Winnowing never evolved within a mouthbrooding clade. Mouthbrooding was also rarely lost: we found no transitions from mouthbrooding to substrate brooding in nonwinnowers and a median of zero transitions from mouthbrooding to substrate brooding in winnowers, with an HPD of zero to two transitions.

We ran the methods described above for two alternate versions of the data set: one where we classified four species with ambiguous reports of mouthbrooding as mouthbrooders (inclusive) and one where we excluded these species entirely (exclusive). Detailed results are provided in

Table 1: Weighted average transition rate matrix between behavioral states

| | Neither | Mouthbrooding | Winnowing | Both |
|---------------|---------|---------------|-----------|------|
| Neither | ... | .33 | 1.76 | .21 |
| Mouthbrooding | .33 | ... | .11 | .11 |
| Winnowing | 1.77 | .11 | ... | 4.85 |
| Both | .21 | .11 | 4.87 | ... |

Note: Shown are transition rate matrices calculated under three different rate assumptions (symmetrical rates, equal rates, and all rates different), averaged on the basis of the Akaike information criterion weights of their respective M_k models (86.9%, 12.8%, and 0.3%, respectively). Rates have been multiplied by 10^3 for readability and so can be interpreted as, for example, $4.8 \times 10^{-3} = 0.0048$ transitions from winnowing to winnowing and mouthbrooding ("Both" column) per 1 million years.

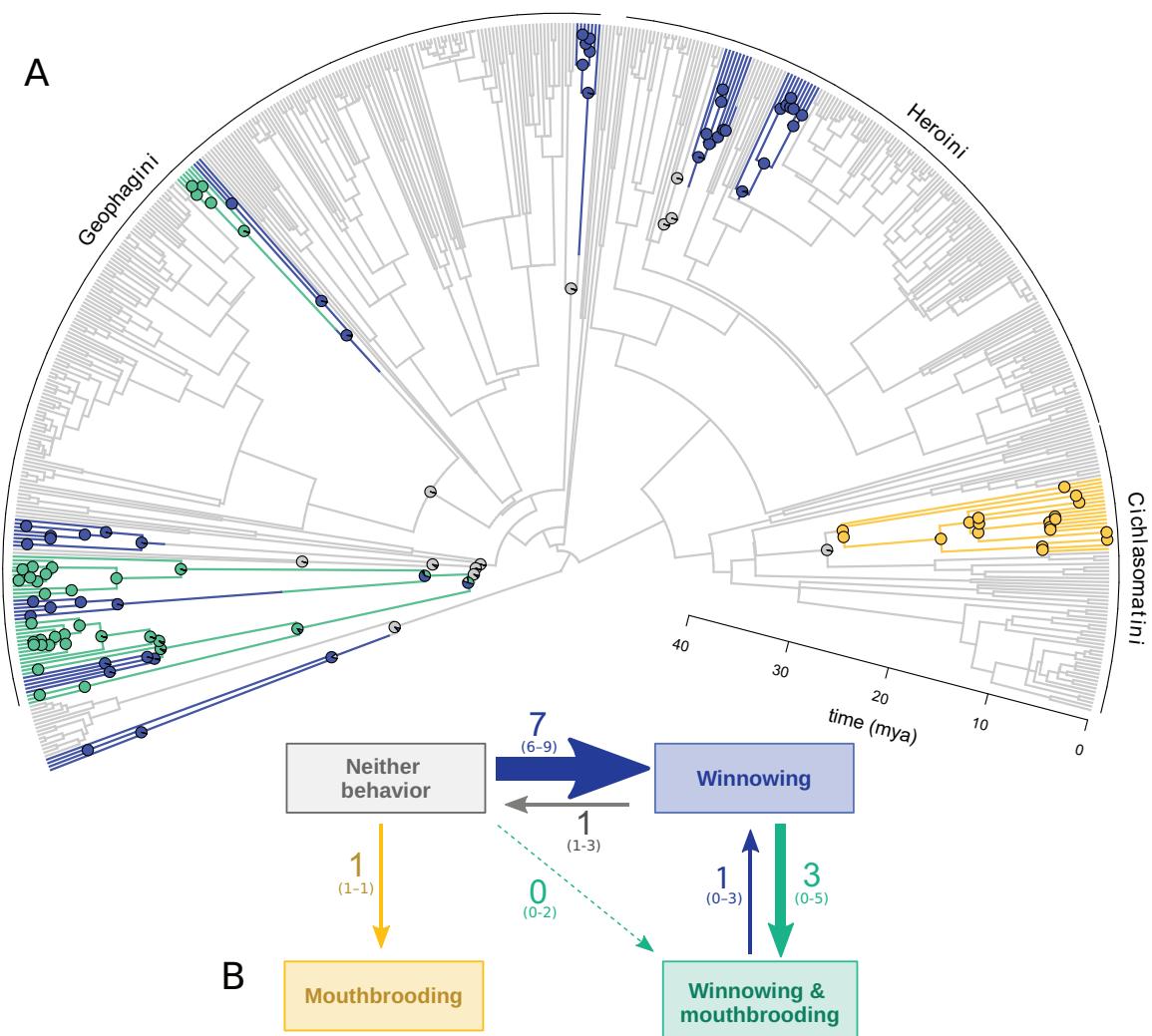


Figure 3: Mouthbrooding and winnowing transitions across Neotropical cichlids. *A*, Transitions between each combination of feeding mode (winnowing and nonwinnowing) and parental care strategy (mouthbrooding and substrate brooding) as an average of 10,000 simulated stochastic character maps (Revell 2012). Pie charts at nodes represent the proportion of character maps for which each state was reconstructed at that node; nodes that were reconstructed as nonmouthbrooding and nonwinnowing in 100% of simulations are not shown. *B*, State transitions across simulations, including average number of transitions (large text) and high-probability density range (in parentheses).

the supplemental PDF, but the results in these cases were qualitatively similar to those we report here: (1) we found the best support for Pagel's (1994) test for a model where mouthbrooding depends on winnowing; (2) we found the highest support for a symmetrical rates M_k model, followed by equal rates and all-rates-different models; and (3) we found that the highest transition rate in the weighted average transition rate matrix was from winnowing to winnowing and mouthbrooding (see sec. S2 of the supplemental PDF).

Buccal Cavity Area Differences

Mouthbrooders and winnowers had larger buccal cavities than species that exhibited neither behavior (fig. 4A). In

nonwinnowing species, mouthbrooders had considerably larger buccal cavities compared with substrate brooders (nearly 60% as a proportion of head area). All species of *Bujurquina*, a genus of mouthbrooding cichlasomatines, had buccal cavities between 11% and 17% of head area; *Andinoacara*, the nearest substrate-brooding sister taxon to *Bujurquina*, had buccal cavities between 6% and 9% of head area. Among winnowing species, both mouthbrooders and substrate brooders had large buccal cavities (12%–24% of head area for mouthbrooding winnowers and 10%–21% of head area for substrate-brooding winnowers).

We used a phylogenetic ANOVA (Adams and Collyer 2018) to test whether buccal cavity areas depended on feeding mode and parental care strategy, fitting buccal cavity area

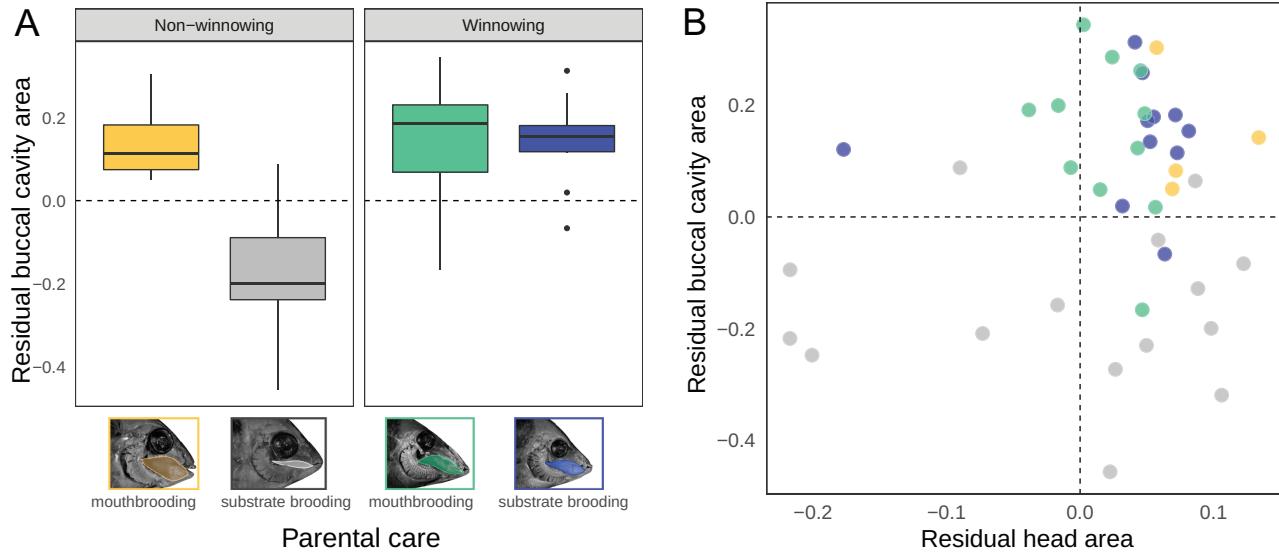


Figure 4: Buccal cavity areas in Neotropical cichlids across feeding and parental care categories. *A*, Residual buccal cavity area (after phylogenetic body size correction) in mouthbrooding and substrate-brooding nonwinnowers and in mouthbrooding and substrate-brooding winnowers. Representative species with highlighted buccal cavities are shown on the *x*-axis (from left to right: *Bujurquina hophrys*, *Mesonauta insignis*, *Satanoperca jurupari*, and *Cribroheros robertsoni*). *B*, Same values and color scheme as in *A*, plotted against residual head area.

as a function of parental care mode and feeding mode, with squared standard length included as a covariate to control for body size. We found that only feeding mode had a significant effect at the $\alpha = .05$ threshold ($P = .015$), while the effect of parental care mode was considerably weaker ($P = .14$), as was their interaction term ($P = .15$). The effect of parental care was significant when we performed a nonphylogenetic ANOVA ($P = .0002$), as was the interaction of parental care and feeding mode ($P = .003$). We found the same results using the phylogenetic residuals from the phyl.resid function as an alternative method of correcting for body size. This indicated that the magnitude of difference between buccal cavity areas was substantial but that because we have a single clade of nonwinnowing mouthbrooders in our sample, we lack sufficient phylogenetic resolution to attribute that difference to mouthbrooding.

To test whether these buccal cavity area differences were largely driven by differences in head area across taxa, we also fit a PGLS model of buccal area as a function of head area, again with squared standard length as a covariate. We found no significant relationship between buccal cavity area and head area ($P = .19$; fig. 4B). We also tested whether head area differed significantly across behavioral categories by fitting a PGLS model of head area as a function of parental care and feeding mode, again with standard length as a covariate. We found no significant relationship between head area and any of the variables ($P > .35$ for feeding, parental care, and their interaction).

Buccal Cavity Shape Differences

Both mouthbrooders and winnowers had similar buccal cavity shapes, even after controlling for size. In a principal components analysis, PC1 (47% of shape variance) corresponded largely to buccal cavity depth (fig. 5A), with both mouthbrooding and winnowing species having deeper buccal cavities. Differences in parasphenoid curvature accounted for most of the change in buccal cavity depth. More highly curved parasphenoids result in a greater distance between the roof and the floor of the buccal cavity, producing a larger overall volume even when the mouth is fully closed. PC2 (24% of shape variance) largely separated different substrate-brooding nonwinnowing taxa from each other, while winnowers and mouthbrooders spanned a relatively narrow range of PC2 scores.

We found that only feeding mode, not parental care or their interaction, had a significant effect on buccal cavity shape in a PGLS model ($P < .01$), although this result can be partially attributed to group aggregation on the phylogeny (see “Discussion”). We also used the PGLS model to predict buccal cavity shape for each of the four behavioral categories (fig. 5C) and calculated Procrustes distances between each pair of shapes to quantify shape differences. The highest Procrustes distance (least similar) between any two predicted shapes was between the mouthbrooding winnower shape and the buccal cavity shape for neither behavior. The lowest distance (most similar) was between the

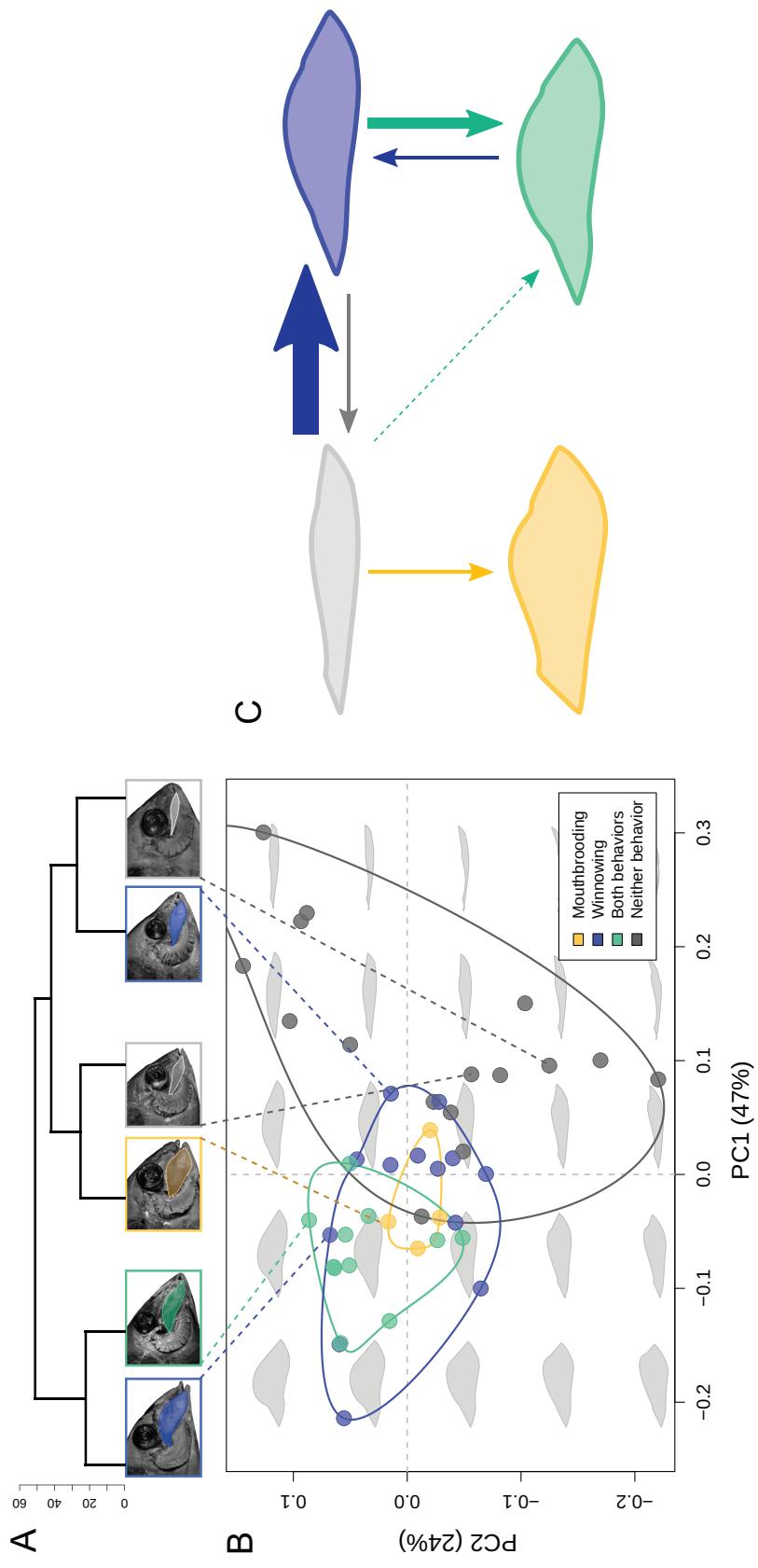


Figure 5: Variation in buccal cavity shape across feeding and parental care categories. *A*, Buccal cavity shapes among selected sister taxa belonging to different behavioral categories. From left to right: *Satanopercajurupari* (mouthbrooding winnower) and *S. daemon* (substrate-brooding winnower) and *Andinoacara pulcher* (substrate brooder), and *Bujurquinaophrys* (mouthbrooder) and *Andinoacara pulcher* (substrate brooder). *B*, First two principal components (PCs) of buccal cavity shape for all species in the data set. Points represent species averages. Back-transforms were calculated using the method described in Olsen (2017). *C*, Predicted shapes for each behavioral category from the phylogenetic generalized least squares model, with arrows proportional to the number of transitions between each state, as shown in figure 3*B*.

mouthbrooding and substrate-brooding winnower shapes, followed by the mouthbrooder and winnower shapes.

Discussion

Our results support two major conclusions: (1) among Neotropical cichlids, mouthbrooding transitions occur at a much higher rate in winnowers than in nonwinnowers; and (2) winnowing and mouthbrooding are both associated with a larger, more curved buccal cavity, a phenotype with clear functional implications for each behavior. Taken together, these lines of evidence suggest that mouthbrooding may evolve more frequently in winnowing lineages because winnowing morphologies are also advantageous for mouthbrooding. This provides compelling, if incomplete, support for the co-optation hypothesis for increasing multi-functionality because our results indicate that mouthbrooding is more likely to evolve if anatomical constraints associated with the behavior have been previously relaxed by adaptation to a substrate-sifting feeding mode.

Mouthbrooding Evolves More Frequently in Winnowing Clades

Each discrete modeling method supported the same conclusion: mouthbrooding is gained and lost more frequently in winnowing than in nonwinnowing clades. The results of Pagel's (1994) correlation test strongly support that transitions to or from mouthbrooding occur much more often in winnowing lineages but that transitions to or from winnowing do not depend on parental care. Our Mk model and stochastic character map results also generally favored mouthbrooding being gained within winnowers more often than it was lost (fig. 3), although we did recover some ambiguity around the directionality of this change (table 1).

This ambiguity is partly a consequence of our model design, in which we created four discrete character states as a combination of parental care and feeding states. It makes more biological sense that winnowing and mouthbrooding would not be gained simultaneously, but this transition is not penalized by any of our Mk models or stochastic character maps. Incorporating this assumption into future models could clarify the most likely direction of this transition, but even without making an assumption either way, our analyses are highly consistent overall: mouthbrooding transitions occur more often in winnowers.

We also noted that the combination of winnowing and mouthbrooding has only evolved within the Geophagini tribe (in *Gymnogeophagus*, *Geophagus* sensu stricto, the “*Geophagus*” *steindachneri* group, and *Satanoperca*). This could be a function of clade age: geophagines are much older as a group than the Heroini and evolved winnowing much

earlier. It could also be a simple matter of body size, since many of the winnowing geophagines get fairly large (for cichlids), so the potential reduction in total brood volume for mouthbrooding is not as severe on an absolute scale (Steele and López-Fernández 2014). Still, geophagines make up about half of Neotropical cichlid diversity and are substrate brooding in the majority, making our observations generally informative for mouthbrooding evolution in the group.

Both Mouthbrooding and Winnowing Are Associated with Larger Buccal Cavities

We found that both winnowers and mouthbrooders tended to have larger, more curved buccal cavities than nonwinnowers, even relative to their overall head area. This is especially apparent in *Bujurquina* when compared with its nearest substrate-brooding sister taxon (*Andinoacara*): despite extremely similar external cranial morphologies, *Bujurquina* buccal cavities were about twice the size of those in *Andinoacara*, a difference that was mostly achieved via a steeper angle of the ceratobranchial and a higher arch of the parasphenoid, creating more space even when the mouth was closed. While winnowers and mouthbrooders showed clear departures from the buccal cavities of fishes that exhibit neither behavior, mouthbrooding winnowers differed little from their substrate-brooding counterparts (fig. 5). In contrast with *Bujurquina* and *Andinoacara*, mouthbrooding winnowers were nearly indistinguishable from their nearest substrate-brooding sister taxa in each case (compare *Satanoperca jurupari* and *S. daemon* in fig. 5A).

One explanation for these observations is that the mouthbrooding *Bujurquina* + *Tahuantinsuyo* clade evolved larger buccal cavities because these were advantageous for mouthbrooding, while within the winnowing lineages buccal cavities underwent no change when mouthbrooding evolved because winnowing adaptations for winnowing had already resulted in a morphology advantageous for mouthbrooding. This could make sense from a functional morphological perspective. Winnowing involves sifting mouthfuls of substrate for edible material, probably by controlling fluid flow in the mouth to manipulate substrate particles, a process punctuated by cycles of premaxillary protrusion, mouth opening, and opercular flaring (Weller et al. 2017). Mouthbrooding, at least in Neotropical cichlids, is a superficially similar process: eggs (and sometimes fry) are tumbled in the mouth via repeated cycles of premaxillary protrusion, hyoid depression, and opercular flaring (Van Wassenbergh et al. 2016).

Both processes require holding, sensing, and manipulating many particles in the buccal cavity and having fine control over whether those particles are swallowed, with the meaningful difference that in winnowing the goal is

to swallow those particles whereas in mouthbrooding the goal is to keep the offspring tumbling in the mouth while the parent abstains from feeding. Mouthbrooders swallowing their brood (filial cannibalism) is frequently reported in other mouthbrooding groups (Okuda 2000), including in African cichlids, where it is apparently accidental (females often swallow their first brood before successfully rearing their second, per Taborsky 2006). To our knowledge, filial cannibalism has never been reported for a Neotropical mouthbrooding cichlid, although there is comparatively less research on Neotropical mouthbrooding generally. Based on our conjecture here, we would hypothesize that it occurs less frequently in winnowing than in nonwinnowing species.

Having a larger, more rounded buccal cavity not only provides more room for substrate (or eggs) but may also provide some hydrodynamic advantage in generating turbulence and associated water flow around the particles (Brooks et al. 2018). In their experiments with mouthbrooder egg development, Shaw and Aronson (1954) found that eggs not exposed to flowing water developed slowly or not at all, which they attributed to a lack of adequate oxygen diffusion; eggs that were exposed to flowing water but not tumbled (i.e., not freely rotated and translated) were still susceptible to fungal growths. Greater curvature in the parasphenoid produces a buccal cavity shape that resembles a pipe that expands into a sphere in the center: the rapid change in diameter should disrupt laminar flow and produce turbulence, which could allow eggs to spend more time tumbling and less time settled at the bottom of the mouth (Van Wassenbergh et al. 2015; Vogel 2020). Substrate-brooding cichlids generate a constant water flow around their eggs by “fanning” with their fins and bodies, supporting the idea that those conditions would need to be re-created in the mouth cavity of mouthbrooders.

While the results we present here have a number of clear functional implications, a major caveat is that several of our inferences rest on the single clade of confirmed nonwinnowing mouthbrooders among Neotropical cichlids. We can say with some confidence that mouthbrooding and substrate-brooding winnowers have similarly large, curved buccal cavities, but we have only one phylogenetically independent observation of the buccal cavity of a nonwinnowing mouthbrooder in this clade. *Bujurquina*'s larger buccal cavity may be an adaptation to mouthbrooding, but it may be due to another selective pressure (like respiration or feeding) or even the product of a constructional constraint (Barel 1983). This could potentially be addressed by expanding our data set to include putative Neotropical mouthbrooders, such as *Aistogramma megastoma* (Römer et al. 2017); the large mouth for which this species is named may have a correspondingly large buccal cavity. Expanding the scope of our phylogenetic analysis to include mouthbrooding cichlid clades from Africa should also reveal

whether the morphological attributes of winnowing mouthbrooders and the rates of transition between them are similar to those we found in the Neotropics.

However, even if we can attribute this increased buccal cavity area to mouthbrooding, convergent morphologies between two behaviors are not conclusive evidence of an evolutionary path of least resistance leading from one to the other. Both winnowing and mouthbrooding are dynamic processes, involving constant shape changes to the notoriously kinetic fish head; a wide variety of buccal cavity shapes could be achieved by adjustments to posture alone, and cranial expansion (as during suction feeding) probably contributes more to increased buccal cavity volume than any change to static morphology possibly could. The morphologies of these fishes are at best indirect proxies for their behaviors. In that sense, it is somewhat remarkable that we recovered the magnitude of differences that we present here and that these differences appear to be specific to the buccal cavity (as opposed to the overall head area). A more complete understanding of the relationship between feeding and mouthbrooding will require a detailed study of the behavioral, kinematic, and mechanical components of each process and of how and where they might overlap or conflict.

When and How Much Does Morphology Matter for Mouthbrooding?

The results we present here support the hypothesis that, at least in Neotropical cichlids, mouthbrooding evolves more often from winnowing than it does from other feeding modes because winnowing produces a feeding phenotype that is advantageous for mouthbrooding. We can construct a plausible evolutionary sequence of events from this interpretation: a lineage evolves winnowing, a feeding mode that selects for, among other traits, a large, curved buccal cavity and the necessary motor control and sensation for manipulating particles intraorally. These traits are adaptive for winnowing but happen to be advantageous for mouthbrooding as well. If a fish in this lineage begins mouthbrooding, even facultatively, it is likely to have much more success than a fish from a nonwinnowing lineage would because it can mouthbrood a larger brood volume for longer periods of time. By comparison, a nonwinnowing fish without these feeding traits must have either a smaller brood (fewer or smaller eggs) or a higher rate of offspring loss to physiological limits, such as inadequate oxygenation to the eggs in the buccal cavity. In this context, mouthbrooding is advantageous for the winnower but not the nonwinnower, and it is selected for only in the winnowing population.

Such a model would explain the higher transition rate to or from mouthbrooding in winnowing lineages, but

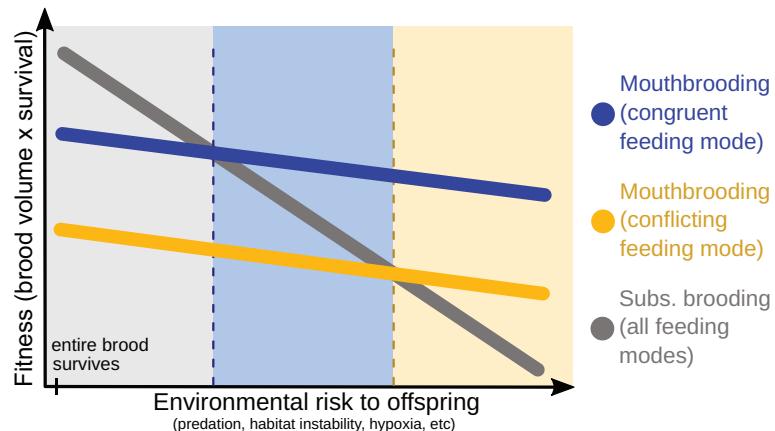


Figure 6: Proposed model for how feeding morphology affects parental care evolution. *A*, Fitness (number of surviving offspring) is a product of brood size and brood survival, represented by the filled rectangles for each level of environmental risk. Mouthbrooding (blue and yellow points) reduces the rate of offspring loss to some kinds of environmental risks compared with substrate brooding, at the fixed cost of a smaller maximum brood volume. *B*, As environmental risks increase, mouthbrooding yields more surviving offspring than substrate brooding. Species with congruent feeding modes can incubate a larger brood, meaning that mouthbrooding becomes selectively advantageous in a wider range of environments.

it is an incomplete explanation. Mouthbrooding clearly evolves even in the absence of a phenotypic alignment with an existing feeding mode. Among Neotropical cichlids, the *Bujurquina* + *Tahuantinsuyoa* clade is the best-documented example of nonwinnowing mouthbrooders, but there are reports from the aquarium hobby of facultative mouthbrooding in *Heros liberifer* (Staeck and Schindler 2015) and at least two species of *Aistogramma*, *A. barlowi* (Römer and Hahn 2008) and *A. megastoma* (Römer et al. 2017), both of which have remarkably large heads relative to other members of the group. And among the Central American Heroini cichlids, winnowing has evolved at least twice (fig. 3), while mouthbrooding has apparently never evolved in this group. Still, this is further complicated in non-Neotropical cichlids, where mouthbrooding is extremely widespread (especially in the African lakes) among taxa that occupy a wide range of feeding niches and morphologies (Liem 1973; Goodwin et al. 1998; McGee et al. 2020). These African mouthbrooders lay fewer eggs than substrate brooders (sometimes by an order of magnitude), and these eggs are considerably larger, with effective diameters averaging twice those of substrate brooders (Coleman 1991, 1998)—meaning that they have even fewer offspring for the same brood volume. Data on egg size and number is much more scarce for Neotropical cichlids, but reports from the aquarium hobby suggest that egg size differences are negligible, although we would require empirical data to draw more reliable conclusions (Coleman and Galvani 1998).

We suggest a conceptual life history model to reconcile these seemingly contradictory observations in which the relative selective advantage of mouthbrooding depends on

both feeding morphology and environmental risks that impact offspring survival (fig. 6). Mouthbrooding is hypothesized to be advantageous for specific environmental correlates of offspring mortality, such as high predation rates, low nest site availability, and hypoxia risk (Östlund-Nilsson and Nilsson 2004; Rüber et al. 2004; Taborsky and Foerster 2004; Duponchelle et al. 2008). As these factors increase, so does offspring mortality, even for mouthbrooders (Vranken et al. 2019). The rate of offspring loss, however, should depend on parental care strategy, with substrate brooders losing proportionally more offspring than mouthbrooders (fig. 6, compare slopes of gray and yellow lines), while mouthbrooders have a lower maximum brood volume. This would suggest a tipping point where mouthbrooding yields more surviving offspring than substrate brooding, but only past some threshold of environmental risk (fig. 6, yellow dashed line).

In this model, feeding phenotype—insofar as it influences cranial morphology and behavior—matters because it determines maximum brood volume. A winnowing species (or one with a similarly congruent feeding mode) should be able to brood a larger volume of offspring than an otherwise identical nonwinnowing species, meaning that mouthbrooding is advantageous for winnowers across a wider range of environments (fig. 6, blue line and blue dashed line). Essentially, when environmental drivers of offspring mortality are extremely low, substrate brooding is advantageous for all feeding modes, and when they are extremely high, mouthbrooding is advantageous for all feeding modes; but for some range of environments, mouthbrooding is advantageous only for species with congruent feeding modes.

Although simple, this conceptual model does yield some testable predictions. First, we would predict that the selection pressure for mouthbrooding is strong in environments where we observe mouthbrooders with conflicting feeding modes (African lakes), intermediate in environments where we mostly observe mouthbrooders with congruent feeding modes (South America), and weak in environments where we observe winnowers but never mouthbrooders (Central America). The relative rates of offspring loss under different environmental conditions would probably be extremely difficult to measure, partly because many of these parameters are unwieldy to collect for a wide range of species and partly because of the number of ecological, biogeographical, and phylogenetic factors influencing life history evolution across these disparate environments.

Much more tractable are two predictions about the costs of mouthbrooding: (1) the reduction in brood size for mouthbrooders (relative to substrate brooders) should be different for winnowers and nonwinnowers and (2) winnowers should have better mouthbrooding performance or lower energetic costs of mouthbrooding than nonwinnowers. The parallel evolution of smaller broods and larger eggs has been well documented in the evolution of mouthbrooding in the African Great Lakes (Duponchelle et al. 2008); to our knowledge, this pattern has never been examined in the Neotropical cichlids. Assessing mouthbrooding performance would first require a biologically reasonable definition of performance in the context of parental care, but measuring this performance in winnowing and nonwinnowing mouthbrooders could provide a fascinating basis for testing whether winnowers really make for superior mouthbrooders.

Constraint, Opportunity, and Multifunctionality

The multifunctional perspective views individual traits as compromises between conflicting functional pressures (Pigliucci 2007). Bird beak shape, for example, must balance foraging, thermoregulation, and song production (Friedman et al. 2019), and Stayton (2019), in his study of hard-shelled turtles, was even able to determine the relative importance of hydrodynamic performance, strength, and self-righting on the evolution of shell shape. Mouthbrooding, too, could be interpreted as a straightforward example of a multifunctional constraint, where conflicting reproductive and feeding pressures reduced cranial morphological diversity (Hoey et al. 2012). Instead, we show that mouthbrooding may be most likely to evolve precisely when it does not introduce additional constraints to the head, possibly in an example of co-optation (or exaptation, per Gould and Vrba 1982). While this does not necessarily contradict the multifunctional perspective, we think it raises a larger evolutionary question: when multifunctionality increases for one

trait, what happens to evolution at the level of the whole organism? We predict that by further constraining one trait, organisms can access new phenotypic space in the evolution of another trait. Mouthbrooding, for example, may release formerly constrictive reproductive constraints, allowing parents to reproduce under a wider range of environmental conditions. In the context of co-optation, increasing multifunctionality may generate more opportunities than constraints—but this is obvious only when studies account for how multifunctional traits affect evolution at the level of the whole organism.

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Statement of Authorship

H.I.W., C.D.M., H.L.-F., and E.L.B. conceptualized the study. H.I.W. and E.L.B. acquired funding. H.I.W. and H.L.-F. determined methods and sampling scheme. H.I.W. collected, analyzed, and visualized the data and wrote the original draft of the manuscript. H.I.W., C.D.M., H.L.-F., and E.L.B. revised and edited the manuscript. C.D.M., H.L.-F., and E.L.B. provided resources (specimens and equipment).

Data and Code Availability

All data and code, including dissection images, landmarks, simulations, and full specimen list, are available in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.4f4qrjdc>).

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