

Parent-offspring conflict, ecology, and life history diversification of livebearing fishes

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Major shifts in life history evolution—such as the transition from pre- to post-fertilization maternal provisioning in viviparous organisms—require complex morphological and physiological adaptations. The conditions under which these transitions arise remain largely unexplored. Theory postulates that parent-offspring conflict can drive diversification in maternal provisioning strategies, but while alternative hypotheses focusing on sexual selection and ecological adaptation have been proposed, neither have been evaluated comparatively. Livebearing fishes (Poeciliidae) have provided important insights into life history evolution due to variation in maternal provisioning strategies, mating systems, and ecology. Comparative analyses of temporal dynamics across 94 species revealed that repeated shifts from lecithotrophy (pre-fertilization provisioning) to matrotrophy (post-fertilization provisioning) preceded concomitant changes in sexual selection, establishing a previously missing causal link in the correlated evolution of life history and mating systems. Adaptive hypotheses of matrotrophy evolution were supported by correlations between ecological variables and the extent of post-fertilization provisioning. Consistent with theoretical models, matrotrophy was associated with environments exhibiting high primary productivity and low competition. In contrast, results did not support strictly conflict-driven scenarios of matrotrophy evolution. Bi-directional evolution along the lecithotrophy-matrotrophy continuum contradicted the expectation of intraspecific Red Queen dynamics with perpetual increases in post-fertilization provisioning. Our results demonstrate that parent-offspring conflict alone is not sufficient to explain the evolution of matrotrophy, but may play a role in conjunction with ecological mechanisms. We propose that natural selection mediated by resource availability counterbalances conflict-fueled—potentially maladaptive—increases in matrotrophy.

Evolutionary transitions in maternal provisioning strategies represent a primary axis of reproductive life history variation in viviparous organisms (1, 2). Shifts from an ancestral strategy of females providing all resources for embryonic development prior to fertilization (lecithotrophy) to post-fertilization provisioning (matrotrophy) have been associated with the evolution of complex physiological and morphological adaptations, including placental structures of apposed maternal and embryonic tissues that facilitate nutrient transfer (3). Matrotrophy and placentas have evolved repeatedly in viviparous animals, including multiple invertebrate (4-8) and vertebrate lineages (9-12). Transitions along the lecithotrophy-matrotrophy continuum may have far reaching consequences, shaping the evolution of other traits and patterns of biological diversification. The exacerbation of parent-offspring conflict associated with the evolution of matrotrophy (13, 14) has been hypothesized to impact patterns of sexual selection and promote speciation (15-18). What evolutionary forces shape the evolution of matrotrophy consequently is a pivotal question in evolutionary biology (19).

Two conceptual frameworks may explain the evolution of matrotrophy. (1) The viviparity-driven conflict hypothesis postulates that matrotrophy evolves as a consequence of parent-offspring conflict over the degree of maternal investment, leading to antagonistic coevolution between maternal and embryonic traits that impact nutrient transfer during pregnancy (17, 20). This hypothesis predicts positive feedback loops between matrotrophy and the strength of parent-offspring conflict, and variation in maternal provisioning strategies is not expected to be related to environmental variables (16, 19, 20). The viviparity-driven conflict hypothesis also predicts correlated evolution between life history and sexual selection, because matrotrophy can facilitate post-copulatory mate choice by females and weaken the importance of pre-copulatory sexual selection and male courtship (15). While there is empirical evidence for correlated evolution between matrotrophy and sexual selection (15), the causal relationship between life history evolution and

sexual selection remains unresolved. It has been assumed that shifts in maternal provisioning strategies drive shifts in sexual selection, but the directionality of this relationship may just as likely be reversed (21, 22). Evolution along a continuum of mating systems ranging from those characterized predominantly by male coercion to those predominantly exhibiting male courtship and pre-copulatory female choice may favor diversification in life history strategies, where females of species exhibiting high rates of male coercion should be selected for reduced pre-fertilization provisioning to avoid costs associated with genetically incompatible sires. (2) Matrotrophy may be a consequence of adaptive evolution in response to ecological sources of selection. Matrotrophy has been hypothesized to reduce locomotor costs associated with pregnancy (23-25). Specifically, lecithotrophic females are expected to suffer from impaired locomotion throughout gestation, while matrotrophic females with initially small embryos should avoid such costs at least in early stages of pregnancy (26, 27). In addition, resource availability may shape evolution along the lecithotrophy-matrotrophy continuum. Lecithotrophy is expected to be adaptive in environments with fluctuating resource availability (27), whereas matrotrophy theoretically maximizes reproductive output when resource availability is high and stable (28).

Livebearing fishes of the family Poeciliidae have become an iconic model system for testing hypotheses about the evolution of reproductive strategies (29). Poeciliids have undergone remarkable diversification in levels of post-fertilization maternal provisioning [with independent origins of matrotrophy in different clades (15, 19, 30)], as well as male mating strategies and sexual selection [ranging from mating systems dominated by male coercion to systems dominated by pre-copulatory female mate choice (15, 31, 32)]. In addition, species in this family are broadly distributed throughout the Americas, found in a wide variety of ecological contexts, and have a well-resolved phylogeny (33-35), facilitating comparative analyses that contrast alternative hypotheses about the evolutionary origins of matrotrophy. Research on poeciliid fishes has been instrumental for the

advancement of our theoretical and empirical understanding of matrotrophy (19, 27, 36), and there is circumstantial evidence supporting various predictions of both the viviparity-driven conflict and adaptive hypotheses of matrotrophy evolution. Intra-specific analyses have shown that the evolution of matrotrophy is associated with parent-offspring conflict (37, 38), and a comparative study documented the predicted correlation between matrotrophy and sexual selection (15). Furthermore, pregnancy in poeciliids is associated with locomotor costs expected under the adaptive hypothesis (39, 40), and there is evidence that matrotrophy increases streamlining (41) and is favored in high predation environments requiring efficient escape responses (42). Finally, maternal provisioning also responds to resource availability (43, 44), and matrotrophy is associated with significant costs when resources fluctuate (45).

Here, we used phylogenetic comparative analyses of 94 species spanning the family Poeciliidae to test predictions of the viviparity-driven conflict and adaptive evolution hypotheses to gain insights into ultimate drivers of matrotrophy evolution: (1) The viviparity-driven conflict hypothesis predicts correlated evolution between life history and sexual selection, but the causal relationship remains unclear. We used phylogenetic time-series analyses to test whether life history evolution drives sexual selection, or vice versa. (2) The viviparity-driven conflict hypothesis predicts that positive feedbacks lead to continuous increases in matrotrophy through evolutionary time. We used ancestral state reconstruction to evaluate the directionality of matrotrophy evolution during the diversification of poeciliids. (3) The adaptive hypothesis of matrotrophy evolution predicts correlations between ecological variables and the degree of matrotrophy. We used phylogenetic path analyses to test whether variation in abiotic and biotic environmental variables relevant to locomotor costs and resource availability predict variation along the lecithotrophy-matrotrophy continuum.

Results

Evolution of matrotrophy shapes sexual selection, not vice versa

A prior study documented correlated evolution of matrotrophy and sexual selection in poeciliid fishes (15), but the causal relationship was not resolved. Three hypotheses could explain correlated evolution: (1) Shifts along the lecithotrophy-matrotrophy continuum (driven by parent-offspring conflict or ecological selection) may drive the evolution of sexual selection (15, 16); (2) evolution in patterns of sexual selection along a continuum from male coercion with cryptic female choice to male courtship with pre-copulatory female choice may favor different maternal provisioning strategies and impact parent-offspring conflict (21, 38); or (3) the same (or correlated) environmental factors may exert selection on multiple traits, causing correlated phenotypic evolution without pleiotropic interactions among trait suites.

These alternative hypotheses result in different predictions about time-lags between shifts in the evolution of matrotrophy and sexual selection, which can be contrasted by explicitly testing the relative timing of evolutionary shifts in the two traits across a phylogenetic tree. We quantified variation along the lecithotrophy-matrotrophy continuum based on embryonic mass changes during development [resulting in a matrotrophy index (30)] and variation in sexual selection based on the degree of sexual dimorphism [resulting in a sexual selection index (31)]. Analyses of evolutionary time-lags across 250 trees revealed the strongest support for the model in which shifts in matrotrophy preceded shifts in sexual selection (mean Akaike weight: 0.430; 95% CI: 0.411 – 0.450). The null model (no lag between the two traits) received significantly lower support (mean Akaike weight: 0.312; 95% CI: 0.304 – 0.320), and the model assuming that shifts in sexual selection preceded shifts in matrotrophy had the lowest support (mean Akaike weight: 0.258; 95% CI: 0.244 – 0.272). These results support the previously held assumption that evolution in matrotrophy mediates subsequent changes in sexual selection (15), rejecting the alternative hypothesis that sexual selection was a key driver of life history diversification.

Matrotrophy has evolved bi-directionally during the diversification of poeciliids

A critical assumption of the viviparity-driven conflict hypothesis is that there are positive feedback-loops between parent-offspring conflict and matrotrophy (19). Matrotrophy evolution within species should therefore be characterized by Red Queen dynamics (46). Following the emergence of matrotrophy, coevolution of maternal and embryonic traits that impact nutrient transfer during pregnancy are expected to continuously increase post-fertilization provisioning (20). Consequently, the viviparity-driven conflict hypothesis predicts that lecithotrophy is the ancestral provisioning strategy in the family Poeciliidae, and that unidirectional evolutionary changes have occurred from lecithotrophy toward matrotrophy.

Ancestral state reconstructions (ASR) were used to compare inferred ancestral states of matrotrophy to variation in matrotrophy observed in extant taxa. ASRs across 250 trees demonstrated that lecithotrophy is not the ancestral provisioning strategy (Fig. 1A), challenging previous assumptions about matrotrophy evolution in poeciliids (19). The inferred ancestral state was clearly toward the matrotrophic end of the spectrum (with a net weight gain during development) and distinctly above the levels of post-fertilization provisioning observed in most extant taxa (Fig. 1B). This does not mean that matrotrophy evolved prior to lecithotrophy, but rather that the common ancestor of extant poeciliids had already evolved some degree of post-fertilization provisioning. More importantly, examining the direction of shifts in post-fertilization provisioning strategies between all nodes and their descendants revealed that reductions of matrotrophy were just as common as increases in matrotrophy (Fig. 1C). The bidirectionality of matrotrophy evolution does not support the central prediction of the viviparity-driven conflict hypothesis that matrotrophy should continue to increase throughout diversification.

Ecological variables are correlated with matrotrophy evolution

Ecological selection related to locomotor performance and resource acquisition are the two leading adaptive hypotheses for diversification of maternal provisioning strategies. The locomotor cost hypothesis predicts that matrotrophy should evolve in ecological contexts that select for high locomotor efficiency, for example under high water flow conditions or in habitats with high predation risk (42, 47). Alternatively, matrotrophy may be favored in environments with high and stable resource availability (28). If ecologically-mediated natural selection contributed to the diversification in maternal provisioning strategies, we would predict correlations between the matrotrophy index and ecological variables associated with locomotor performance, resource availability, or both.

We contrasted a series of hypotheses about the hierarchical relationships among different abiotic and biotic environmental variables and matrotrophy using phylogenetic path analysis (48). Environmental predictor variables included metrics of hydrology (based on the topography of each species' range) and predation (number of predatory fish species overlapping each species' range), which are both relevant in the context of the locomotor cost hypothesis (42, 47). In addition, we used metrics of climate (temperature and precipitation patterns in each species' range), average net primary productivity (NPP), and competition (number of poeciliid species overlapping each species' range), which are relevant in the context of the resource availability hypothesis (28). The matrotrophy index and environmental predictor variables were combined in model sets representing 18 different hypotheses about putative relationships among the variables (Fig. S1). Model selection identified two models with average $\Delta\text{CIC}_c < 2$ across the 250 trees (Fig. S2), including NPP, competition, and predation as predictor variables for variation in matrotrophy. Both models indicated that NPP positively correlated with competition ($r = 0.325$, 95% CI = 0.324 – 0.326 for both supported models), which in turn was negatively correlated with matrotrophy (top model: $r = -$

0.030, 95% CI = -0.034 – -0.025; secondary model: $r = -0.077$, 95% CI = -0.081 – -0.075). In addition, there was a positive relationship between NPP and matrotrophy (top model: $r = 0.024$, 95% CI = 0.022 – 0.025; secondary model: $r = 0.023$, 95% CI = 0.021 – 0.024). Simultaneously visualizing the effects of NPP and competition on matrotrophy indicated that high levels of matrotrophy occurred when NPP was high and competition was low (Fig. 1D). This finding is consistent with the predictions of theoretical models that emphasize resource availability (28). In the best supported model, NPP was also positively correlated with predation ($r = 0.184$, 95% CI = 0.183 – 0.185), which was negatively correlated with matrotrophy ($r = -0.082$, 95% CI = -0.087 – -0.078). Notably, however, the directionality of the relationship between predation and matrotrophy was opposite to the predictions of the locomotor cost hypothesis, which posits that matrotrophy should enhance locomotor performance and be favored in high-predation environments (42). Although the effects sizes in the path analyses were relatively small, they were nonetheless significantly different from zero, indicating that ecology has played a role in matrotrophy evolution.

Discussion

Shifts from pre- to post-fertilization maternal provisioning represent a major axis of life history evolution in viviparous organisms (1, 2), with cascading effects for the evolution of complex traits, sexual selection, and speciation (16, 49, 50). We used phylogenetic comparative analyses of 94 livebearing fishes in the family Poeciliidae to contrast hypothesized mechanisms driving the evolution of post-fertilization maternal provisioning. A key assumption of the viviparity-driven conflict hypothesis is a positive feedback-loop between parent-offspring conflict and matrotrophy, resulting in a co-evolutionary arms-race between maternal and offspring traits (19, 20). Conflict mechanisms—by definition—are therefore only sufficient to explain the evolution of matrotrophy if post-fertilization maternal provisioning increases through time. Our analyses indicated that decreases

in levels of matrotrophy were just as common as increases (Fig. 1C). Such bi-directional evolution along the lecithotrophy-matrotrophy continuum has been documented in other viviparous taxa (e.g., 47, 51) and parallels secondary losses of obligate viviparity in fishes and reptiles (52, 53). The standard model of linear life history evolution from oviparity to lecithotrophic viviparity to matrotrophic viviparity (e.g., 17) therefore needs reevaluation to acknowledge that the evolution of these traits is much more complex and dynamic than generally appreciated. Our results demonstrate that evolutionary mechanisms other than parent-offspring conflict must have played a role in the diversification of maternal provisioning strategies of poeciliids.

Correlations between ecological variables and matrotrophy support adaptive hypotheses of matrotrophy evolution. Phylogenetic path analysis identified three biotic variables that were associated with variation in matrotrophy (resource availability, competition, and predation), all of which are well-documented drivers of life history diversification in animals (54-56). While the relationship between matrotrophy and predation was opposite to the predictions of the locomotor cost hypothesis, high levels of matrotrophy coincided with low competition and high resource availability (Fig. 1D). This finding is consistent with the Trexler-DeAngelis model (28), which predicts matrotrophy evolution in environments with abundant and stable resources.

There are two caveats that highlight the inconsistencies between existing conceptual frameworks and empirical data associated with matrotrophy evolution. (1) Although viviparity-driven conflict alone cannot explain matrotrophy evolution due to repeated evolutionary transitions towards lower levels of post-fertilization provisioning ($\Delta MI < 0$ in Fig. 1C), we cannot exclude the possibility that viviparity-driven conflict played a key role in repeated transitions towards matrotrophy [$\Delta MI > 0$ in Fig. 1C (15, 30)]. (2) There is clear empirical evidence that high levels of matrotrophy are disadvantageous when resources are scarce or unpredictable, impacting both offspring size and maternal body condition (45, 57). But while models suggest that matrotrophic

provisioning should outcompete a lecithotrophic strategy when resource availability is high (28), key assumptions of the model do not hold up to empirical scrutiny (57), and we still lack any empirical evidence indicating that matrotrophy provides fitness benefits over lecithotrophy under those conditions (19).

We propose a novel paradigm of matrotrophy evolution that addresses existing caveats by emphasizing interactions between conflict-driven and ecological mechanisms of selection. Specifically, natural selection mediated by resource availability may counterbalance evolutionary trends driven by parent-offspring conflict. Under low resource conditions, incipient matrotrophy comes with substantial costs (28, 45), preventing the escalation of coevolutionary arms-races between maternal and offspring traits, and thus, the evolution of matrotrophy is impeded. Under favorable conditions with stable and abundant resources, matrotrophy may not actually provide a selective advantage over lecithotrophy. Rather, increases in post-fertilization provisioning may be rendered selectively neutral (or merely less maladaptive) under relaxed resource constraints, allowing parent-offspring conflict to fuel increases in matrotrophy. Bi-directional evolution along the lecithotrophy-matrotrophy continuum could be a simple consequence of a shifting balance between ecological selection favoring lecithotrophy and conflict-driven increases in matrotrophy, in a manner similar to how natural selection counter-balances sexually selected traits evolving under Fisherian runaway scenarios (58, 59). The suggested paradigm is consistent with evidence of ecological sources of selection modulating the outcomes of conflict in other contexts, including sexual conflict (60, 61) and sibling conflict (62, 63). Consequently, we may have to consider that matrotrophy is not actually adaptive [see (57)], but that maladaptive increases in post-fertilization provisioning may spread in populations when selection against it is weak. The notion that matrotrophy—as a widespread life history phenomenon—may be a maladaptive trait is also consistent with the observation that evolutionary conflicts commonly cause deviations from adaptive peaks (64). Escalation of parent-

offspring conflict under relaxed constraints is readily possible, because fish eggs have an ancestral ability to acquire organic molecules from the environment through active transport (65); *i.e.*, conditions facilitating potentially maladaptive increases in matrotrophy are present in poeciliids and do not have to evolve *de novo*.

A more rigorous understanding of matrotrophy evolution in the future will require the exploration of interactive effects among evolutionary mechanisms. Combining theoretical models and microevolutionary analyses will allow comparative evaluation of hypotheses about organismal performance under different environmental conditions to explicitly determine the conditions that are conducive to the spread of post-fertilization provisioning under adaptive and maladaptive evolutionary scenarios. The grand question for future research is whether natural selection alone can explain the evolution of post-fertilization provisioning (if there is a consistent—yet unknown—adaptive benefit to matrotrophy), or whether matrotrophy evolution is a consequence of a shifting balance between adaptive and potentially maladaptive evolutionary processes.

Methods

Taxon sampling and phylogenetic framework

Our analyses included 94 species (Table S1), encompassing representatives of all major genera in the family Poeciliidae. These species span a geographic range from the eastern United States south to Argentina, including Caribbean islands (Fig. S3). The phylogenetic framework used for analyses came from previously published studies with a similar taxon sampling (15, 31). In brief, sequences for six mitochondrial (*12S*, *COI*, *CytB*, *ND2*, *tRNA^{Val}*, and *tRNA^{Leu}*) and 11 nuclear genes (*Beta Actin*, *CCND1*, *ENC1*, *GLYT*, *MYH6*, *RAG1*, *Rhodopsin*, *RPS7*, *SH3PX3*, *T36*, and *XSRC*) were obtained from GenBank, aligned, and maximum likelihood phylogenetic analysis was conducted using RAxML-HPC version 8 (66) on the CIPRES computer cluster (San Diego State University,

San Diego, CA, USA). The resulting best scoring tree was highly consistent with previously published phylogenetic hypotheses for the family Poeciliidae (15, 34, 35). Phylogenetic trees were time calibrated with three calibration points spanning the depth of the phylogeny (see 31 for details), including a primary fossil calibration associated with the split separating the outgroup (*Fundulus*) from all poeciliids (55-99 Ma; 67) and a secondary fossil calibration separating *Heterandria formosa* from the genus *Poecilia* (9.3-19 Ma; 68). In addition, the formation of Laguna de Catemaco (Mexico), a crater lake with several endemic species, was used as a constraint on the age of the endemic *Poeciliopsis catemaco* (0.5-2.0 Ma; 69). Even though bootstrap support values on the best scoring tree were generally strong, phylogenetic comparative methods described below were conducted across 250 trees drawn at random from the bootstrap replicates to account for phylogenetic uncertainty.

Matrotrophy, sexual selection, and ecological variables

Matrotrophy index: The extent of post-fertilization maternal provisioning was quantified using the matrotrophy index (MI, ln-transformed for all analyses). The matrotrophy index is the ratio of offspring mass at birth to the mass of the egg at fertilization (19). Offspring of lecithotrophic species typically lose 25-55% of the initial egg mass during development ($MI < 0.75$), while continuous nutrient transfer from mother to offspring during gestation in matrotrophic species leads to $MI > 1$, with some species giving birth to offspring that are up to 100 times heavier than the initial egg (19, 30). Matrotrophy levels for all species included in the analysis were obtained from previously published studies (15, 70).

Sexual selection index: Variation in sexual selection within poeciliids was quantified using a morphology-based sexual selection index, which quantifies the degree of sexual dimorphism in body shape and multiple fin traits that reflect variation in female choice and male mating strategies (31). The index describes variation along a previously established continuum of poeciliid mating systems

(32, 71, 72), ranging from species characterized by long gonopodia and coercive mating tactics (negative index scores) to species with short gonopodia and courtship [positive index scores (31)].

Hydrology, climate, and net primary productivity: Assembly of hydrological and climate variables associated with each species' range was based on georeferenced occurrence points. We obtained 73,398 locality points from multiple sources (<http://fishnet2.net/>, <http://gbif.org/>, primary literature), representing the known distributions for all 94 species included in our study. We first removed duplicate points and retained a maximum of 100 randomly sampled localities within the native range of each species, which is sufficient to capture environmental variation even in wide-ranging species (73). We further verified that all data points for a given species were at least 1 km apart to match the spatial resolution of environmental data. Any locality that did not meet this criterion was either removed for species with <100 localities or replaced by another randomly drawn locality for species with >100 localities. For all locality records, we then extracted values for 19 climatic (Worldclim: <http://worldclim.org/>) and three hydrological variables (elevation, slope and compound topographic index; Hydro1k: <https://lta.cr.usgs.gov/HYDRO1K/>) as well as an estimate of net primary productivity (<https://lpdaac.usgs.gov/>) at a spatial resolution of $\sim 1 \text{ km}^2$ (30 arcsec) in ArcMap version 10.2.2 (ESRI Inc, Redlands, CA, USA). For each species, we calculated the median value for all 23 variables. Climatic and hydrological variables were then subjected to separate phylogenetic principal component analyses (pPCA) using a correlation matrix, as implemented in the PHYTOOLS package in R (74). In addition to the estimate of net primary productivity associated with each species' range, we retained two pPCA axes accounting for 66 % of variation in hydrology (Table S2) and three pPCA axes accounting for 81 % of variation in climate (Table S3).

Competition and predation: Quantifying the actual biotic interactions for a large number of species distributed across the vast geographic scale included in this study is virtually impossible, and

we developed two simple, objectively quantifiable metrics to approximate levels of competition and predation. We assumed that competitive interactions for the focal species primarily occur with other species of the family Poeciliidae (75, 76) and that the intensity of competition is a function of the number of coexisting species. Hence, we first analyzed overlap of distributional ranges to characterize patterns of sympatry [defined as range overlap values greater than zero (77)]. We created geo-referenced distributional range maps for each species by generating a convex hull around each species' known occurrence points (see above) using ArcMap. The resulting species-specific distributions were then intersected to determine the total number of competitor species exhibiting a range overlap with a focal species. Similarly, we created a metric estimating the levels of predation by determining the total number of piscivorous fish species exhibiting a range overlap with a focal species. To do so, we obtained 271,148 locality points (<http://gbif.org/>) of 7,170 species across 1602 genera and 26 families in the superclass Osteichthyes that coincide with the distribution of poeciliids. Since distributional polygons of some focal species overlapped with marine habitats (particularly in poeciliid species occurring along the Gulf of Mexico and in both island and mainland localities), we first removed species primarily inhabiting marine environments as well as non-native species, retaining 5019 native freshwater species in 853 genera (Table S4). To identify potential predators, we conducted a genus-level literature search of dietary habits using relevant monographs (78-82), supplemented by the primary literature when necessary. We retained 131 genera that included species with evidence for piscivory (867 species, 73,421 locality points). Values for the number of competitors and predators were square-root-transformed prior to analyses.

Analytical approaches

Evolutionary time-lag analysis: We used the MVMORPH package in R (83) to evaluate the causal relationship underlying the previously documented correlated evolution of matrotrophy and sexual

selection. MVMORPH implements Ornstein–Uhlenbeck models to test whether one trait follows the optimum of a second trait with a detectable time-lag. We used the matrotrophy index and the sexual selection index as focal traits, contrasting a null model (no detectable lag) to a model that assumes shifts in matrotrophy lag behind shifts in sexual selection (i.e., sexual selection drives matrotrophy evolution) and a model that assumes shifts in sexual selection lag behind shifts in matrotrophy (i.e., matrotrophy drives evolution of sexual selection). Analyses assuming a unique adaptive optimum (OU1 model) yielded identical results to assuming multiple optima (OUM model). Analyses were conducted across 250 random trees. Models were compared based on their average Akaike weights calculated with a finite sample correction (84).

Ancestral state reconstruction: Ancestral state reconstructions of matrotrophy index were conducted using PHYTOOLS (74). Inferences of ancestral states were averaged across 250 random trees. To evaluate the directionality of matrotrophy evolution between each node and its descendants, we extracted trait reconstructions for each node of the tree and calculated ΔMI as the observed (tip) or inferred (node) matrotrophy values subtracted from the values of its most recent ancestral node.

Phylogenetic path analysis: We investigated hypotheses about the hierarchical relationships among abiotic and biotic environmental factors and matrotrophy using phylogenetic path analysis as implemented in the R package PHYLOPATH (85). We developed 18 models based on *a priori* hypotheses about the effects of climate, hydrology, competition, and predation, as well as interactions between predictor variables relevant in the context of the locomotor cost and resource limitation hypotheses of matrotrophy evolution (Fig. S1). Phylogenetic path analysis was run across 250 random trees. PHYLOPATH implements model selection with covariance inflation criterion [CIC_C (86)]. Models with an average $\Delta CIC_C < 2$ were considered equally supported (87). Joint effects of net primary productivity and competition were visualized using non-parametric thin-plate spline

regression to create a surface of matrotrophy variation (88). Estimation of matrotrophy surfaces was performed using the FIELDS package in R (89) (smoothing parameter $\lambda = 0.005$).

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References

1. Blackburn DG (1992) Convergent evolution of viviparity, matrotrophy, and specializations for fetal nutrition in reptiles and other vertebrates. *American Zoologist* 32:313-321.
2. Wourms JP (1981) Viviparity: the maternal-fetal relationship in fishes. *American Zoologist* 21:473-515.
3. Wooding P & Burton G (2008) *Comparative placentation: structures, functions and evolution* (Springer-Verlag, Berlin).
4. Campiglia SS & Walker MH (1995) Developing embryo and cyclic changes in the uterus of *Peripatus* (*Macroperipatus*) *acacioi* (Onychophora, Peripatidae). *Journal of Morphology* 224:179-198.
5. Hart MW, Byrne M, & Smith MJ (1997) Molecular phylogenetic analysis of life-history evolution in asterinid starfish. *Evolution* 51:1848-1861.
6. Korniushev AV & Glaubrecht M (2003) Novel reproductive modes in freshwater clams: brooding and larval morphology in southeast Asian taxa of *Corbicula* (Mollusca, Bivalvia, Corbiculidae). *Acta Zoologica* 84:293-315.
7. Korneva ZV (2005) Placental type interactions and evolutionary trends of development of uterus in cestodes. *Journal of Evolutionary Biochemistry and Physiology* 41:552-560.

8. Ostrovsky AN, *et al.* (2016) Matrotrophy and placentation in invertebrates: a new paradigm. *Biological Reviews* 91:673-711.
9. Wourms JP, Grove BD, & Lombardi JL (1988) The maternal-fetal relationship in viviparous fishes. *Fish Physiology*, ed Hoar WS (Academic Press, London), pp 2-134.
10. Wake MH & Dickie R (1998) Oviduct structure and function and reproductive modes in amphibians. *Journal of Experimental Zoology* 282:477-506.
11. Steward JR & Blackburn DG (1988) Reptilian placentation: structural diversity and terminology. *Copeia* 1988:839-852.
12. Wildman DE, *et al.* (2006) Evolution of the mammalian placenta revealed by phylogenetic analysis. *Proceedings of the National Academy of Sciences USA* 103:3203-3208.
13. Haig D (1993) Genetic conflict in human pregnancy. *Quarterly Review of Biology* 68:495-532.
14. Haig D (1996) Altercation of generations: genetic conflicts of pregnancy. *American Journal of Reproductive Immunology* 35:226-232.
15. Pollux BJA, Meredith RW, Springer MS, Garland T, & Reznick DN (2014) The evolution of the placenta drives a shift in sexual selection in livebearing fish. *Nature* 513:233-236.
16. Zeh DW & Zeh JA (2000) Reproductive mode and speciation: the viviparity-driven conflict hypothesis. *Bioessays* 22:938-946.
17. Furness AI, Morrison KR, Orr TJ, Arendt JD, & Reznick DN (2015) Reproductive mode and the shifting arenas of evolutionary conflict. *Annals of the New York Academy of Sciences* 1360:75-100.
18. Coleman SW, Harlin-Cognato A, & Jones AG (2009) Reproductive isolation, reproductive mode, and sexual selection: empirical tests of the viviparity-driven conflict hypothesis. *American Naturalist* 173:291-303.

19. Pollux B, Pires M, Banet A, & Reznick D (2009) Evolution of placentas in the fish family Poeciliidae: an empirical study of macroevolution. *Annual Review of Ecology Evolution and Systematics* 40:271-289.
20. Crespi B & Semeniuk C (2004) Parent-offspring conflict in the evolution of vertebrate reproductive mode. *American Naturalist* 163:635-653.
21. Haig D (2014) Sexual selection: placentation, superfetation, and coercive copulation. *Current Biology* 24:R805-R808.
22. Long TAF (2005) The influence of mating system on the intensity of parent-offspring conflict in primates. *Journal of Evolutionary Biology* 18:509-515.
23. Shaffer LR & Formanowicz DR (1996) A cost of viviparity and parental care in scorpions: reduced sprint speed and behavioral compensation. *Animal Behavior* 51:1017-1024.
24. Miles DB, Sinervo B, & Frankino WA (2000) Reproductive burden, locomotor performance, and the cost of reproduction in free ranging lizards. *Evolution* 54:1386-1395.
25. Magnhagen C (1991) Predation risk as a cost of reproduction. *Trends in Ecology and Evolution* 6:183-185.
26. Miller RR (1975) Five new species of Mexican poeciliid fishes of the genera *Poecilia*, *Gambusia*, and *Poeciliopsis*. *Occasional Papers of the Museum of Zoology, University of Michigan* 672:1-44.
27. Thibault RE & Schultz RJ (1978) Reproductive adaptations among viviparous fishes (Cyprinodontiformes: Poeciliidae). *Evolution* 32(2):320-333.
28. Trexler JC & DeAngelis DL (2003) Resource allocation in offspring provisioning: An evaluation of the conditions favoring the evolution of matrotrophy. *American Naturalist* 162(5):574-585.

29. Evans JP, Pilastro A, & Schlupp I eds (2011) *Ecology and Evolution of Poeciliid Fishes* (The University of Chicago Press, Chicago, IL), p 380.
30. Reznick D, Mateos M, & Springer M (2002) Independent origins and rapid evolution of the placenta in the fish genus *Poeciliopsis*. *Science* 298:1018-1020.
31. Culumber ZW & Tobler M (2017) Sex-specific evolution during the diversification of live-bearing fishes. *Nature Ecology & Evolution* 1:1185-1191.
32. Bisazza A, Grapputo A, & Nigro L (1997) Evolution of reproductive strategies and male sexual ornaments in poeciliid fishes as inferred by mitochondrial 16 rDNA gene phylogeny. *Ethology, Ecology & Evolution* 9:55-67.
33. Meffe GK & Snelson FF (1989) An ecological overview of poeciliid fishes. *Ecology and evolution of lifebearing fishes (Poeciliidae)*, eds Meffe GK & Snelson FF (Prentice Hall, New Jersey), pp 13-31.
34. Reznick DN, Furness AI, Meredith RW, & Springer MS (2017) The origin and biogeographic diversification of fishes in the family Poeciliidae. *PLoS ONE* 12:e0172546.
35. Hrbek T, Seckinger J, & Meyer A (2007) A phylogenetic and biogeographic perspective on the evolution of poeciliid fishes. *Molecular Phylogenetics and Evolution* 43:986-998.
36. Pires MN, Banet AI, Pollux BJA, & Reznick DN (2011) Variation and evolution of reproductive strategies. *Ecology and Evolution of Poeciliid Fishes*, eds Evans JP, Pilastro A, & Schlupp I (University of Chicago Press, Chicago, IL), pp 28-37.
37. Schrader M & Travis J (2008) Testing the viviparity-driven-conflict hypothesis: parent-offspring conflict and the evolution of reproductive isolation in a poeciliid fish. *American Naturalist* 172(6):806-817.

38. Ala-Honkola O, Friman E, & Lindström K (2011) Costs and benefits of polyandry in a placental poeciliid fish *Heterandria formosa* are in accordance with the parent–offspring conflict theory of placentation. *Journal of Evolutionary Biology* 24:2600-2610.
39. Plaut I (2002) Does pregnancy affect swimming performance in female mosquitofish, *Gambusia affinis*? *Functional Ecology* 16:290-295.
40. Ghalambor CK, Reznick DN, & Walker JA (2004) Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). *American Naturalist* 164(1):38-50.
41. Fleuren M, Quicazan-Rubio EM, van Leeuwen JL, & Pollux BJA (2018) Why do placentas evolve? Evidence for a morphological advantage during pregnancy in live-bearing fish. *PLoS ONE* 13:e0195976.
42. Gorini-Pacheco B, Zandonà E, & Mazzoni R (2017) Predation effects on matrotrophy, superfetation and other life history traits in *Phalloceros harpagos*. *Ecology of Freshwater Fish* 27:442-452.
43. Marsh-Matthews E & Deaton R (2006) Resources and offspring provisioning: A test of the Trexler-DeAngelis model for matrotrophy evolution. *Ecology* 87:3014-3020.
44. Trexler JC (1997) Resource availability and plasticity in offspring provisioning: Embryo nourishment in sailfin mollies. *Ecology* 78(5):1370-1381.
45. Pollux BJA & Reznick DN (2011) Matrotrophy limits a female's ability to adaptively adjust offspring size and fecundity in fluctuating environments. *Functional Ecology* 25:747-756.
46. Rice WR & Holland B (1997) The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. *Behavioral Ecology and Sociobiology* 41:1-10.

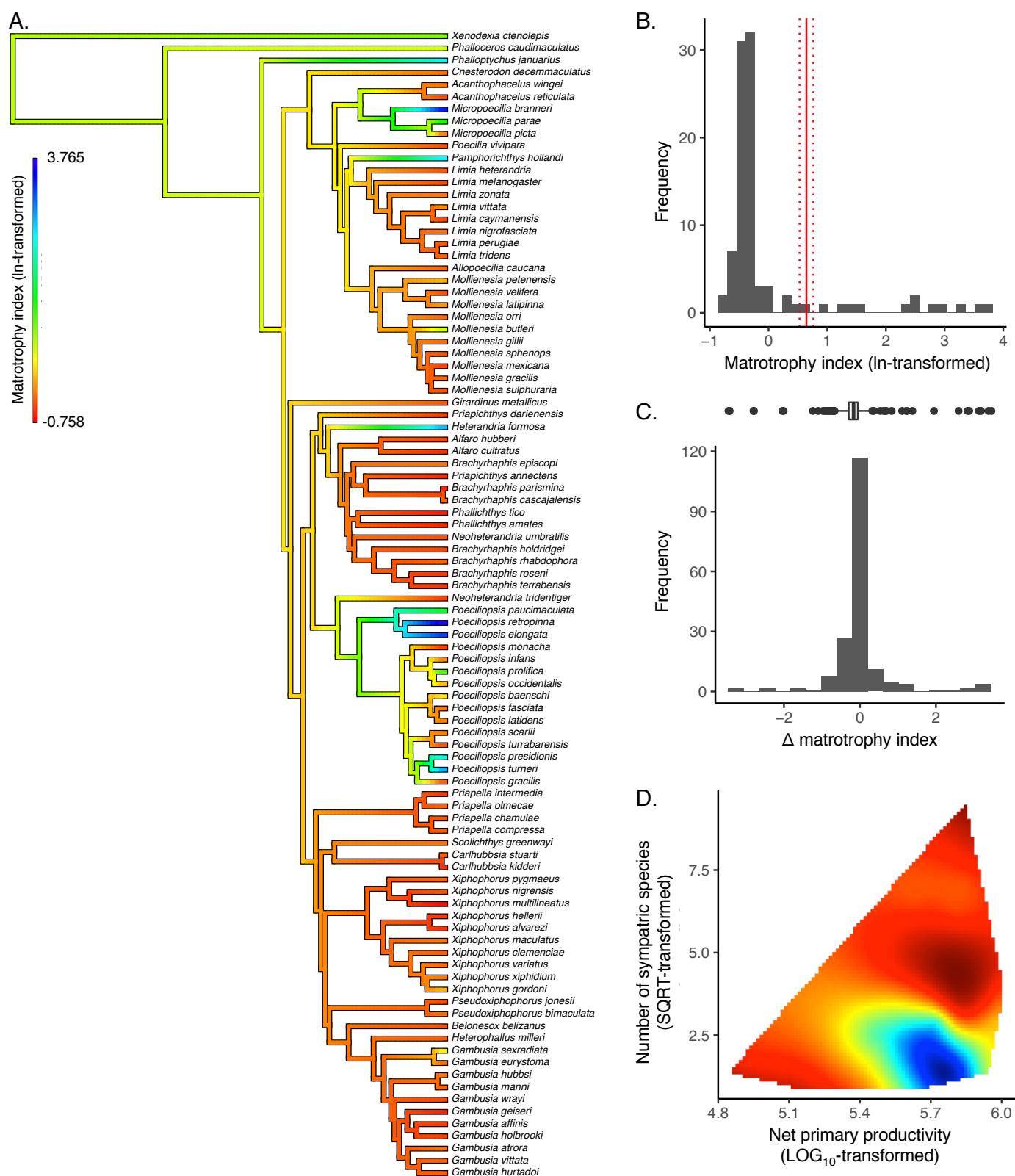
47. Reznick D, Meredith R, & Collette BB (2007) Independent evolution of complex life history adaptation in two families of fishes, live-bearing halfbeaks (Zenarchopteridae, Belontiiformes) and Poeciliidae (Cyprinodontiformes). *Evolution* 61:2570-2583.
48. Gonzalez-Voyer A & von Hardenberg A (2014) An introduction to phylogenetic path analysis. *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology*, ed Garamszegi LZ (Springer, Berlin), pp 201-229.
49. Meredith RW, Pires MN, Reznick DN, & Springer MS (2011) Molecular phylogenetic relationships and the coevolution of placentotrophy and superfetation in (Poecilia) (Poeciliidae: Cyprinodontiformes). *Molecular Phylogenetics and Evolution* 59:148-157.
50. Zeh JA & Zeh DW (2008) Viviparity-driven conflict. *Annals of the New York Academy of Sciences* 1133(1):126-148.
51. Dulvy NK & Reynolds JD (1997) Evolutionary transitions among egg-laying, live-bearing and maternal inputs in sharks and rays. *Proceedings of the Royal Society B* 264:1309-1315.
52. Parenti LR, LoBostro FL, & Grier H (2010) Reproductive histology of *Tomeurus gracilis* Eigenmann, 1909 (Teleostei: Atherinomorpha: Poeciliidae) with comments on evolution of viviparity in atherinomorph fishes. *Journal of Morphology* 27:1399-1406.
53. Recknagel H, Kamenos NA, & Elmer KR (2018) Common lizards break Dollo's law of irreversibility: genome-wide phylogenomics support a single origin of viviparity and re-evolution of oviparity. *Molecular Phylogenetics and Evolution* 127:579-588.
54. Wilson AJ (2014) Competition as a source of constraint on life history evolution in natural populations. *Heredity* 112:70-78.
55. Martin TE (1995) Avian life history evolution in relation to nest sites, nest predation, and food. *Ecological Monographs* 65:101-127.
56. Stearns SC (1976) Life-history tactics: a review of ideas. *Quarterly Review of Biology* 51:3-47.

57. Banet AI, Au AG, & Reznick DN (2010) Is mom in charge? Implications of resource provisioning on the evolution of the placenta. *Evolution* 64:3172-3182.
58. Lande R (1981) Models of speciation by sexual selection on polygenic traits. *Proceedings of the National Academy of Sciences* 78(6):3721-3725.
59. Kirkpatrick M (1982) Sexual selection and the evolution of female choice. *Evolution* 36:1-12.
60. Arbuthnott D, Dutton EM, Agrawal AF, & Rundle HD (2014) The ecology of sexual conflict: ecologically dependent parallel evolution of male harm and female resistance in *Drosophila melanogaster*. *Ecology Letters* 17:221-228.
61. Fricke C, Andersson C, & Arnqvist G (2010) Natural selection hampers divergence of reproductive traits in a seed beetle. *Journal of Evolutionary Biology* 23:1857-1867.
62. Mock DW & Parker GA (1997) *The evolution of sibling rivalry* (Oxford University Press, Oxford).
63. Rubenstein DR (2011) Spatiotemporal environmental variation, risk aversion, and the evolution of cooperative breeding as a bet-hedging strategy. *Proceedings of the National Academy of Sciences USA* 108:10816-10822.
64. Queller DC & Strassmann JE (2018) Evolutionary conflict. *Annual Reviews of Ecology, Evolution and Systematics* 49:73-93.
65. Morrison KR, Ngo V, Cardullo RA, & Reznick DN (2017) How fish eggs are preadapted for the evolution of matrotrophy. *Proceedings of the Royal Society B* 284:20171342.
66. Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9):1312-1313.
67. Santini F, Harmon LJ, Carnevale G, & Alfaro ME (2009) Did genome duplication drive the origin of teleosts? A comparative study of diversification in ray-finned fishes. *BMC Evolutionary Biology* 9(1):1.

68. Ho AFLC, Pruet CL, & Lin J (2016) Phylogeny and biogeography of *Poecilia* (Cyprinodontiformes: Poeciliinae) across Central and South America based on mitochondrial and nuclear DNA markers. *Molecular Phylogenetics and Evolution* 101:32-45.
69. Mateos M, Sanjur OI, & Vrijenhoek RC (2002) Historical biogeography of the livebearing fish genus *Poeciliopsis* (Poeciliidae: Cyprinodontiformes). *Evolution* 56(5):972-984.
70. Olivera-Tlahuel C, Ossip-Klein AG, Espinosa-Pérez HS, & Zúñiga-Vega JJ (2015) Have superfetation and matrotrophy facilitated the evolution of larger offspring in poeciliid fishes? *Biological Journal of the Linnean Society* 116(4):787-804.
71. Martin SB, Albert JS, & Leberg PL (2010) The evolution of the poeciliid gonopodium: integrating morphological and behavioral traits. *Viviparous Fishes II*, eds Uribe MC & Grier HJ (New Life Publications, Homestead, FL).
72. Greven H (2005) Structural and behavioral traits associated with sperm transfer in Poeciliinae. *Viviparous Fishes*, eds Uribe MC & Grier H (New Life Publications, Homestead, FL), pp 145-163.
73. van Proosdij AS, Sosef MS, Wieringa JJ, & Raes N (2015) Minimum required number of specimen records to develop accurate species distribution models. *Ecography*.
74. Revell LJ (2012) phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3:217-223.
75. Torres-Dowdall J, Dargent F, Handelsman CA, Ramnarine IW, & Ghalambor CK (2013) Ecological correlated of the distribution limites of two poeciliid species along a salinity gradient. *Biological Journal of the Linnean Society* 108:790-805.
76. Alberici da Barbiano L, Waller J, & Gabor C (2010) Differences in competitive efficiency between a sexual parasite and its host provide insight into the maintenance of a sperm-dependent vertebrate species. *Journal of Freshwater Ecology* 25:523-530.

77. Weber MG, Mitko L, Eltz T, & Ramírez SR (2016) Macroevolution of perfume signalling in orchid bees. *Ecology Letters* 19:1314-1323.
78. Miller RR, Minckley W, & Norris S (2005) *Freshwater fishes of Mexico* (University of Chicago Press, Chicago).
79. Bussing WA (1998) *Peces de las Aguas Continentales de Costa Rica - Freshwater Fishes of Costa Rica* (Editorial de la Universidad de Costa Rica, San José) 2nd Ed p 468.
80. Greenfield DW & Thomerson JE (1997) *Fishes of the continental waters of Belize* (The University Press of Florida, Gainesville) p 311.
81. van der Sleen P & Albert JS eds (2017) *Field guide to the fishes of the Amazon, Orinoco, and Guianas* (Princeton University Press, Princeton).
82. Boschung HT, Mayden RL, & Tomelleri JR (2004) *Fishes of Alabama* (Smithsonian Books, Washinton, D. C.).
83. Clavel J, Escarguel G, & Merceron G (2015) mvMORPH: an R package for fitting multivariate evolutionary models to morphometric data. *Methods in Ecology and Evolution* 6:1311-1319.
84. Johnson JB & Omland KS (2004) Model selection in ecology and evolution. *Trends in Ecology & Evolution* 19:101-108.
85. van der Bijl W (2018) phylopath: easy phylogenetic path analysis in R. *PeerJ* 6:e4718.
86. Rodriguez CC (2005) The ABC of model selection: AIC, BIC, and the new CIC. *AIP Conference Proceedings* 803:80-87.
87. Burnham KP & Anderson DR (2002) *Model selection and multimodel inference: a practical information-theoretic approach* (Springer, New York, NY).
88. Arnold SJ (2003) Performance surfaces and adaptive landscapes. *Integrative and Comparative Biology* 43:367-375.

578 89. Nychka D, Furrer R, Paige J, & Sain S (2007) fields: tools for spatial data. *R package version*
579 *9.6.*
580



582 Fig. 1. A. Best-scoring maximum likelihood tree of 94 species in the family Poeciliidae. The ancestral
 583 state reconstruction of matrotrophy is mapped onto the phylogeny, with red colors depicting
 584 lecithotrophy and blue colors matrotrophy (as indicated by the color scale of ln-transformed
 585 matrotrophy index values). B. Frequency histogram of the distribution of matrotrophy index values
 586 in extant poeciliid species. The solid red line represents the average ancestral state reconstruction for
 587 the matrotrophy index across 250 random trees with dotted lines indicating the 95% confidence
 588 interval for the estimate. C. Frequency histogram depicting the relative change in matrotrophy index
 589 between all nodes and their descendants. D. Landscape of matrotrophy variation as a function of net
 590 primary productivity and competition. Colors correspond to variation in matrotrophy, as indicated
 591 in the color scale of panel A.