Report

Current Biology

The evolution of the placenta in poeciliid fishes

Highlights

- Placentation has evolved multiple times in the live-bearing fish family Poeciliidae
- We find no consistent family-wide associations between placentation and habitat
- Placental species tend to have superfetation and reduced reproductive allotment
- Placental species tend to have either few large offspring or many small offspring

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In brief

Furness et al. use phylogenetic comparative methods to ask why placentas evolved repeatedly in the live-bearing fish family Poeciliidae. Analyses suggest placental species exhibit two contrasting life histories (few, large offspring or many, small offspring), raising the possibility of different drivers in different lineages.

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Report

The evolution of the placenta in poeciliid fishes

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SUMMARY

How and why complex organs evolve is generally lost to history. The mammalian placenta, for example, was derived from a single common ancestor that lived over 100 million years ago.[1–3] Therefore, the selective factors favoring this complex trait remain obscure. Species in the live-bearing fish family Poeciliidae have independently evolved placentas numerous times while retaining closely related non-placental sister species.[4–7] This provides the raw material to test alternative hypotheses for the evolution of the placenta. We assemble an extensive species-level dataset on reproductive mode, life histories, and habitat, and then implement phylogenetic comparative methods to test adaptive hypotheses for the evolution of the placenta. We find no consistent family-wide associations between placentation and habitat. However, placental species exhibit significantly reduced reproductive allotment and have a higher likelihood of exhibiting superfetation (the ability to gestate multiple broods at different developmental stages). Both features potentially increase body streamlining and enhance locomotor performance during pregnancy, possibly providing selective advantage in performance-demanding environments such as those with high predation or fast water flow. Furthermore, we found significant interactions between body size and placentation for offspring size and fecundity. Relative to non-placental species, placentation is associated with higher fecundity and smaller offspring size in small-bodied species and lower fecundity and larger offspring size in large-bodied species. This pattern suggests that there may be two phenotypic adaptive peaks, corresponding to two selective optima, associated with placentation: one represented by small-bodied species that have fast life histories, and the second by large-bodied species with slow life histories.

RESULTS AND DISCUSSION

How and why do complex traits evolve? We use the placenta as an exemplar to address this question. The placenta is a complex trait that provides the equivalent services of all major organ systems via a physiological linkage between two genetically and immunologically distinct players: mother and offspring.[8,9] The mammalian placenta has been extensively studied from a physiological, anatomical, and immunological perspective,[1,8–10] yet we know little about how and why it evolved because it did so just once >100 million years ago.[1–3] To test hypotheses on placental evolution, we have turned instead to the live-bearing fish family Poeciliidae, which includes maternal provisioning strategies that range from fully provisioning eggs before fertilization (lecithotrophy) to extensive matrotrophy, or the post-fertilization maternal provisioning of embryos during gestation, via a follicular placenta.[4–7] The placenta has evolved at least nine times in this family.[4,6] The existence of placental species closely allied to species that lack placentas, variation in the magnitude of placentation, and multiple, independent origins of placentas enables us to use phylogenetic comparative methods to test alternative hypotheses regarding the factors favoring placental evolution.

Lecithotrophic species begin development with the fertilization of fully provisioned eggs, and then retain developing offspring with no additional maternal provisioning. Embryos lose mass during development at a rate comparable to that of the developing eggs of oviparous fishes.[11] Placental species instead begin development with the fertilization of small eggs. Offspring gain weight over the course of development,[6,12] but there is substantial variation among species in the amount of weight gained.[4–7,12] The matrotrophy index (MI) is a unit-less measure of the degree of post-fertilization provisioning that quantifies this variation.[7] The MI equals the dry mass of fully developed offspring divided by the dry mass of a fertilized egg, so it is a measure of the proportional change in dry mass during development. Lecithotrophic species have MI values <1 because they lose weight during development. Matrotrophic species have MI >1 and may exceed 100 in some species (Figure 1).

There are adaptive (i.e., resource-availability, locomotor-performance, and life-history facilitation) and conflict hypotheses for the evolution of placentas.[6,12] The resource-availability model of Trexler and DeAngelis[14] predicts placentation to be favored in stable high-resource environments because it increases maternal fecundity. The locomotor-performance hypothesis

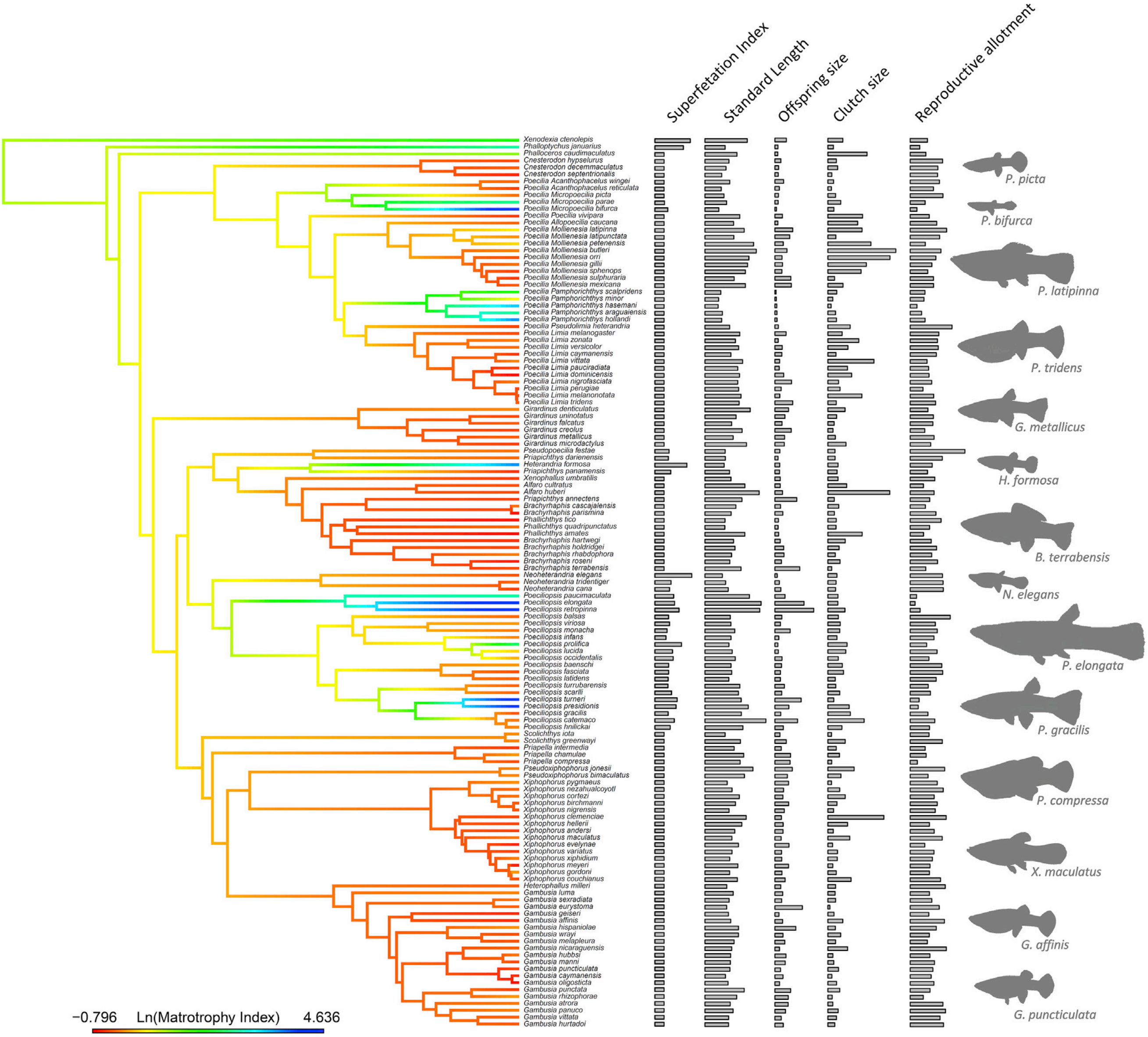

Figure 1. The matrotrophy index reconstructed on the phylogenetic tree of the family Poeciliidae

Branch colors depict a maximum-likelihood reconstruction of maternal provisioning for natural log-transformed matrotrophy indices. The ancestral state reconstruction was performed using the contMap function in the R package phytools.[13] Bars on the tree tips indicate species mean life-history values for the degree of superfetation, standard length, offspring size at birth, number of embryos, and reproductive allotment. Tip silhouettes depict females of representative poeciliid species, scaled to reflect their relative body size. Highly matrotrophic species include some of the largest (i.e., *P. elongata*) and smallest (i.e., *P. bifurca* and *H. formosa*) species in the family, with comparable diversity in life-history traits.

predicts placentation to be favored in performance-demanding environments such as those with fast water flow or high predation,[6,15] because placentation decreases pregnant females' reproductive burden, thus facilitating body streamlining. The life-history facilitation hypothesis predicts that placentation evolves to facilitate the evolution of other features of the life history, such as larger offspring size at birth. The conflict hypothesis instead posits that the placenta evolves as a non-adaptive by-product of an arms race between mothers and gestating offspring over control of resource transfer.[16] The adaptive hypotheses make contrasting predictions regarding the life-history traits associated with placentation and the ecological conditions where placentation is expected to be favored. We combine our species-level dataset on reproductive mode, life histories, and habitat with a robust phylogenetic tree and apply phylogenetic comparative methods to test the predictions and assumptions of the adaptive hypotheses.

Resource-availability hypothesis

Trexler and DeAngelis[14,17] used analytic and simulation models to evaluate the conditions favoring the evolution of placentation. They predicted that fluctuating low-resource environments will hinder the evolution of the placenta unless placental species are able to abort and re-absorb developing offspring when

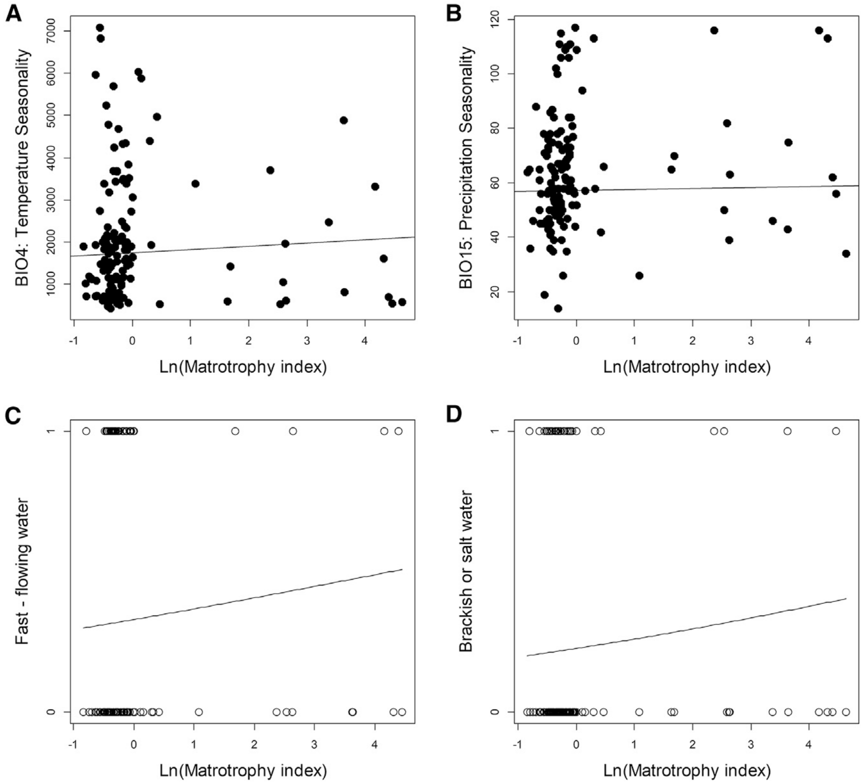

Figure 2. The relationship between matrotrophy and climate/habitat across poeciliid fishes

Phylogenetic generalized least-squares analyses show no relationship ($p > 0.05$) between the matrotrophy index and proxies of resource stability including (A) temperature seasonality and (B) precipitation seasonality. Likewise, phylogenetic logistic regression analyses show no relationship ($p > 0.05$) between the matrotrophy index and inhabitation of (C) fast-flowing water and (D) brackish or salt water. See also Figures S1 and S2 and Tables S1 and S2.

conditions deteriorate. Experimental studies of species that represent four independent origins of placentas reveal that females are not able to abort offspring in response to reductions in food availability during gestation,[18–21] so placental species require a steady source of nutrition to provision offspring. In contrast, offspring of lecithotrophs require no further nutritional provisioning during development so they are not at the mercy of an unreliable maternal food supply during pregnancy. Trexler and DeAngelis[14,17] thus predict that placentation is most likely to evolve in stable, high-resource environments. We extracted a standard set of 19 climate variables[22] from the GPS coordinates where each species in our dataset has been collected to provide proxies for the degree of seasonal fluctuation. We found no significant associations between the MI and any climate variables, including the two that are likely the best indices of seasonal variation: temperature and precipitation seasonality (Figures 2A, 2B, and S1; Table S2). A subsidiary prediction is that placental species may be found in a more restricted set of habitats (have smaller geographic ranges), whereas lecithotrophs may be more cosmopolitan and widespread (larger geographic ranges). Our logic is that placental species may be restricted to stable high-resource habitats, which are a subset of habitats populated by lecithotrophic species. We found no relationship between the MI and geographic or altitudinal range (Figure S2; Table S2).

The Trexler and DeAngelis model assumes that the fitness advantage of placentation is increased fecundity, given that all other life-history components (body size, reproductive allotment, offspring size) are held constant. We assembled the most comprehensive species-level life-history dataset to date from dissections of museum and private collections. We utilized this dataset to test whether placental species exhibit higher fecundity than lecithotrophic species, while controlling for body size, and found that placental species do not have higher fecundity than lecithotrophs across the entire range of body sizes in the family (Figure 3C). Our analyses at this scale thus do not support the predictions of the resource-availability hypothesis.[14]

Locomotor-performance hypothesis

The locomotor-performance hypothesis proposes that placentation is an adaptation that reduces a mother's reproductive allocation (RA) during pregnancy, thereby improving "streamlining" and providing a selective advantage in performance-demanding environments.[6,15] In placental species, resources are transferred to offspring throughout development, rather than provisioned up-front as fully provisioned eggs. One consequence of placentation is that RA—the proportion of total mass that consists of developing young and reproductive tissues—is reduced, especially early in pregnancy.[6,23–27] We found the MI is indeed negatively correlated with RA (Figure 3A). One potential caveat is that RA was calculated using the dry weights of tissues. The relationship is not significant when based on wet weights (Figure S2; Table S3). It is unclear whether this represents a real result or an artifact of the substantially lower sample size for wet weight estimates.

We found a significant positive relationship between the MI and superfetation (Figure 3B). Superfetation (the ability of females to simultaneously gestate multiple broods of offspring at different stages of development) is another reproductive adaptation predicted to reduce peak RA and therefore facilitate streamlining.[6,12,28,29] Superfetation effectively divides a single large litter into multiple smaller litters produced more frequently. It reduces average offspring size present at any point in time and thus reduces the maximum RA.[6] Our phylogenetic analysis reveals that the evolution of either superfetation or placentation facilitates the gain of the other trait, so that the two are likely to appear together (Figure 4).

Strong water flow velocity has been implicated as an important selective factor favoring fusiform bodies.[29,30] A net reduction in RA tied to the evolution of placentation presumably increases streamlining, thereby enhancing swimming performance.[6,12,15,24]

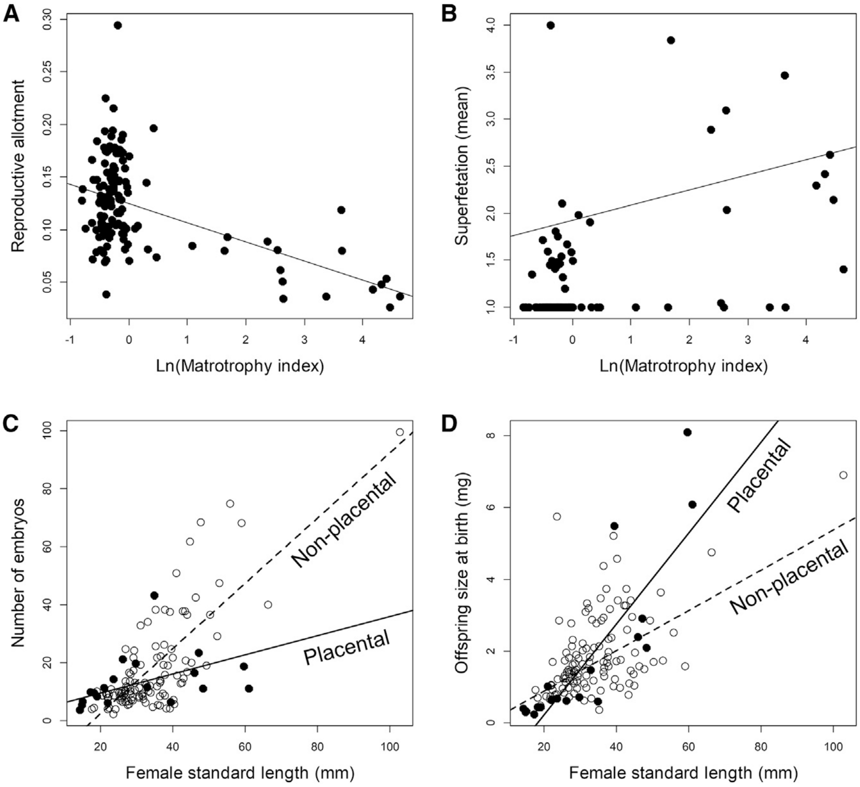

Figure 3. The relationship between matrotrophy and life-history traits across poeciliid fishes

Phylogenetic generalized least-squares analyses show a significant negative relationship ($p < 0.05$) between the matrotrophy index and reproductive allotment (A), and a significant positive relationship ($p < 0.05$) between the matrotrophy index and the superfetation index (B). In (C) and (D), we classify species as placental (closed circles, solid line) and non-placental (open circles, dashed line) to visualize the nature of the significant ($p < 0.05$) interaction between the continuous matrotrophy index and female standard length on the number of embryos (C) and offspring size at birth (D). See also Figure S2 and Tables S1 and S3.

The locomotor-performance hypothesis links this consequence of placental reproduction to environments that favor streamlining.[6] We tested whether the MI is related to whether a species inhabits fast-flowing water and found no such relationship (Figure 2C; Table S2). High-predation environments could also be considered performance demanding because reduced RA could enhance escape response.[31,32] We used (low) elevation and inhabitation of brackish or salt water as proxies for predation because such habitats have higher species diversity,[33] which is generally associated with higher predation intensity. We found no significant relationship between the MI and inhabitation of brackish or salt water (Figure 2D; Table S2), or between the MI and either the mean or maximum altitude of occurrence (Figure S2; Table S2).

The locomotor-performance hypothesis[6,15] fares better than the resource-availability hypothesis, but we do not find the predicted association between placentation and occurrence in environments in which streamlining and swimming performance are expected to be under strong selection. However, our coarse measure of water velocity may not capture variation in species found in a variety of water bodies and ignores microhabitat usage, which can enable species to limit the current they are exposed to (A. A. Hagmayer, A.I.F., and B.J.A.P., unpublished data). Our proxies of predation level are also coarse, plus many species range across a variety of predation habitats.[34] An alternative test of this prediction could be made among populations within a species that ranges across different habitats. A recent study of 27 populations of the highly placental *Poeciliopsis retropinna* showed that those that co-occur with predators had significantly higher MI values than those from sites without predators. This increase in the MI resulted in lower RA, which is predicted to increase body streamlining and facilitate escape performance.[35]

Life-history facilitation hypothesis

The life-history facilitation hypothesis is a catch-all for multiple proposals that the placenta evolved to facilitate the evolution of some other feature of the life history[23,26] such as larger litter size, larger offspring size at birth, improved survivorship early in life, or earlier maturity.[3,15,36–42] These hypotheses are often based on patterns in one or a few species. Addressing them means testing for associations between individual life-history traits, such as offspring size, and matrotrophy. We found no consistent associations of any feature of the life history (other than superfetation and RA, reported above) with the MI (Figure S2; Table S3). However, we did find significant interactions between the MI and standard length for fecundity (Figure 3C) and offspring size at birth (Figure 3D). Relative to lecithotrophic species, small-bodied placental species exhibit numerous small offspring, whereas large-bodied placental species exhibit few large offspring. Thus, the predicted relationship between placentation and larger offspring size is supported only in large-bodied placental species and the predicted relationship between placentation and fecundity is supported only in small-bodied species.

These interactions encapsulate a pattern that is apparent across the family. Some of the smallest species (i.e., *Heterandria formosa*, *Poecilia bifurca*, *Phalloptychus januarius*, *Poeciliopsis prolifica*) with the fastest life histories are placental, as are some of the largest species (*Poeciliopsis elongata*, *Poeciliopsis retropinna*, *Poeciliopsis paucimaculata*, *Poeciliopsis presidionis*, *Xenodexia ctenolepis*) with the slowest life histories. These alternatives are mirrored in the northern and southern clades of *Poeciliopsis*, each of which represents the independent origin of the placenta.[23,26] In the northern clade, placentation is associated with a fast life history[26] relative to lecithotrophic sister species, whereas in the southern clade, placentation is associated with a slow life history.[23] The only consistent life-history trait correlated with the MI across both replicates is a reduction in RA.[23] This pattern of contrasting life-history strategies suggests

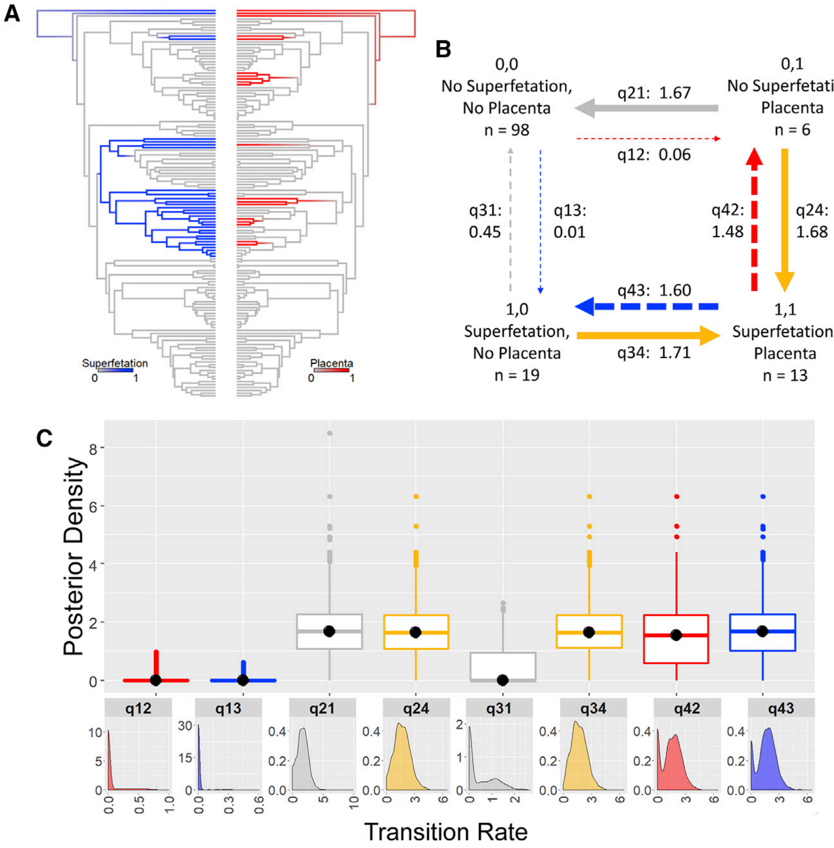

Figure 4. The correlated evolution of superfetation and the placenta

(A) Superfetation (blue) and placentation (red) plotted on the phylogeny using stochastic character mapping in the R package phytools.[13]

(B) Summary diagram of the transition rates across the four combinations of character states from the BayesTraits Discrete dependent model of evolution. The sample sizes for each combination of character states are reported. The arrows are scaled to reflect the magnitude of mean transition rates from the posterior distribution, with the mean value also indicated. Arrows are dashed when the given parameter is equal to zero in >5% of models from the posterior.

(C) Posterior distributions of the transition rates from the Discrete dependent model are shown as boxplots for comparison and as posterior density plots for each transition rate alone. In the boxplots, the black dot indicates the median, the box indicates the upper and lower quartiles, the vertical line indicates the 95% credible intervals of the posterior distributions, and the filled dots beyond the lines indicate outliers.

that there may be two adaptive phenotypic (life-history) peaks associated with placentation, which in turn suggests that the placenta evolved for different reasons in different lineages.

Interaction among hypotheses

Hypotheses for the evolution of placentation may not be mutually exclusive,[6] and the same explanation may not apply to all instances of placental evolution; e.g., placentation may facilitate locomotion in large-bodied species but enhance fecundity in stable resource-rich environments in small-bodied species. Phylogenetic comparative methods generally presume that the same explanation applies to every transition. If the placenta evolves for different reasons in different lineages, then we might expect contrasting trait associations among lineages, rather than a consistent signal. Here, we entertain the hypothesis that placentation evolves in two settings for different reasons: (1) lowland high-resource and high-predation environments where placentas facilitate a fast life history, and (2) fast-flowing rivers favoring streamlining during pregnancy and large size at birth to facilitate offspring locomotion. We tested whether the lack of consistent association between the MI and habitat is due to small-bodied and large-bodied placental species living in two contrasting habitat types. We found that the best-fitting phylogenetic generalized least-squares (PGLS) models did not include these non-significant interactions between the MI and body size (Figures S1 and S2; Table S2). Therefore, ecological associations do not appear to explain the presence of placental species with contrasting life histories.

If different selective factors have favored placentation in different lineages, then we might also expect alternative adaptive peaks of co-adapted trait constellations. We implemented adaptive landscape analyses that test for the existence of such alternative peaks.[43–45] The R package SURFACE[43,44] and l1ou[45] employ the Ornstein-Uhlenbeck model of stabilizing selection[46,47] to identify convergent evolution of phenotypic peaks in continuous character space across a phylogenetic tree using a stepwise information criterion framework. These methods complement our PGLS analyses because they employ the same phylogenetic tree and phenotypic characters. However, instead of specifying a model to be fit to the data, adaptive landscape analyses determine the optimal number of phenotypic shifts using an iterative forward phase (i.e., peak addition) and backward phase (i.e., collapse of convergent peaks). We implemented SURFACE and l1ou using six life-history traits—Ln (MI), superfetation index, standard length, offspring size, offspring number, and RA. We did not find evidence for two alternative convergent placental life-history peaks—one for large-bodied species with few large offspring and another for small-bodied species with numerous small offspring. Instead, most matrotrophic species or clades occupied a unique life-history peak (Figures S3 and S4; Table S4). This pattern may be caused by the over-splitting of matrotrophic species on the basis of the MI and superfetation index. Thus, we consider the idea that matrotrophic species occupy a limited number of life-history optima, a hypothesis deserving of further testing.

Conclusions

We tested three adaptive hypotheses for the repeated evolution of the placenta across the family Poeciliidae. Our results do not support all the predictions of any one hypothesis. In particular, we found some support for predictions of the locomotor-performance hypothesis, namely placentation being associated with superfetation and a reduced RA, both of which are predicted to facilitate streamlining. However, we did not find any association between the MI and climate or habitat variables. If placentation is adaptive, then why is this the case? One possibility is that the ancestral environment in which placentation evolved is different from where these species currently are found. Alternatively, matrotrophic and lecithotrophic species may occupy different niches—in terms of diet, microhabitat usage, and other environmental features—that are not reflected in our choice of climate and habitat variables that characterize their environments. Finally, although framed as alternatives, these hypotheses may not be mutually exclusive. Our finding that placentas are associated with contrasting life-history axes (e.g., small body size and many, small offspring versus large body size and few, large offspring) suggests that there may be multiple environments that favor the evolution of placentas.

However, there is a non-adaptive hypothesis to consider: the viviparity-driven conflict hypothesis.[16] Conflict over resource transfer between mother and offspring derives from relatedness asymmetries.[48] Each offspring shares all genes with itself yet shares only half its genes with its mother and full siblings, whereas a mother is equally related to each of her offspring. Thus, mothers should allocate resources equally to all offspring but offspring maximize their fitness by acquiring a larger than average share of maternal resources.[49] The intimate contact between mother and offspring in live-bearing species creates the potential for offspring to extract maternal resources during gestation.[16,50] We have documented what could be a first step in this interaction by showing that egg-laying relatives of the Poeciliidae can actively absorb small organic molecules at a rate comparable to lecithotrophic poeciliids.[51] Any such foothold of offspring influence can initiate a repeated cycle of adaptation and counter-adaptation between mother and offspring over resource transfer, driving the evolution of placentation.[16] According to the conflict hypothesis, placentation evolves as the by-product of this conflict rather than as an enhancement of maternal fitness.[16,52] The fate of such conflict depends on whatever genetic, physiological, and anatomical variation is present in each lineage. It is not expected to show any particular link to ecological conditions.[16] Therefore, the absence of associations between the MI and features of the environment might reflect the influence of conflict-driven evolution. Finally, conflict and adaptive evolution may potentially interact. Conflict may be important in the initial development of matrotrophy, providing the substrate on which adaptation can occur. Alternatively, the adaptive evolution of matrotrophy may exacerbate mother-offspring conflict driving the elaboration of the placenta once this provisioning strategy takes hold.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2021.02.008.

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AUTHOR CONTRIBUTIONS

A.I.F., with input from D.N.R. and J.C.A., designed the study. A.I.F., Y.R., and D.N.R. collected the data. A.I.F. conducted analyses and B.J.A.P., J.C.A., and D.N.R. contributed to interpretation of the results. A.I.F. and D.N.R. wrote the manuscript with input from all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
BayesTraits V3	[53]	http://www.evolution.rdg.ac.uk/BayesTraitsV3.0.1/BayesTraitsV3.0.1.html
R Project for Statistical Computing	R Core Team	http://www.r-project.org/
phytools	[54]	https://cran.r-project.org/web/packages/phytools/index.html
phylolm	[55]	https://cran.r-project.org/web/packages/phylolm/index.html
ape	[56]	https://cran.r-project.org/web/packages/ape/index.html
caper	[57]	https://cran.r-project.org/web/packages/caper/index.html
surface	[45]	https://cran.r-project.org/web/packages/surface/index.html
l1ou	[47]	https://github.com/khabbazian/l1ou

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Andrew Furness (afurness001@gmail.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The datasets and code are available at Mendeley Data: https://doi.org/10.17632/n7f9cyhxc5.1

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The family Poeciliidae contains 276 species of live-bearing fish. Reproductive mode and life-history data for 160 species was obtained from preserved specimens (i.e., pregnant females) derived from museum (AMNH, ANSP, FMNH, KU, MCP, MNRJ, MZUSP, TCWC, TNHC, TU, UCR, UF, UFBA, UFRGS, UFRJ, UIST, UMMZ, and USNM) and personal collections. Further information can be found in Method details.

METHOD DETAILS

Dataset

To test hypotheses for the evolution of the placenta, we assembled a species-level dataset composed of four trait categories: reproductive mode, life history, habitat, and climatic niche. Reproductive mode and life-history data have been collected in the laboratory of David Reznick over the past four decades, through standard life-history dissections[7,58] of preserved wild-caught specimens derived from museum and personal collections.[5,54] The raw individual-level life-history data for 505 populations, representing 160 species, was compiled and organized into a single standardized datasheet. We wrote an R script to calculate summary reproductive mode and life-history variables for each population, and from this population-level dataset calculated single species' mean values for each trait, weighted by the number of pregnant females in each population. Reproductive mode included the measurement of two traits: an index of placentation and of superfetation. The extent of placentation was quantified using the matrotrophy index,[4,5,7] a continuous measure of the degree of post-fertilization maternal embryo provisioning. It is defined as the dry weight of fully developed offspring ready to be born (stage 45) divided by the dry weight of unfertilized eggs (stage 0). In practice, this unitless ratio ranged from values as low as 0.5 to 0.7, indicating embryos lose 30 to 50% of their dry weight over the course of development owing to metabolic processes, a degree of weight loss comparable to that seen in the eggs of spawning species,[11] to a high of 103, indicating over 100-fold embryo weight gain during gestation. Species with matrotrophy indices of less than one are considered lecithotrophic or reliant on pre-fertilization yolk stores, while those with larger matrotrophy indices obtain nutrients, and hence gain weight, during pregnancy due to maternal provisioning across a follicular placenta. The matrotrophy index was calculated for each collection by first regressing natural log-transformed embryo dry weight as a function of developmental stage, with embryo developmental stage scored according to standard embryo morphological criteria common to all poecilid species.[59] Embryos below stage 5 were excluded to avoid unintentional inclusion of under-weight stage 0 embryos which were not fully yolked up. The dry weight of stage 0 and stage 45 embryos were then estimated from the regression parameters derived from this linear model. These values were back transformed from the natural log scale, and their ratio used to calculate the collections' matrotrophy index. The superfetation index is the number of broods at distinct developmental stages that a pregnant female carries simultaneously. This ranged from 1 (all embryos at a single developmental stage) to a maximum of 7 broods at distinct stages of development. Female life-history traits included standard length, ovary wet weight, total reproductive tissue dry weight, dry reproductive allotment (total reproductive tissue dry weight / female dry weight), wet reproductive allotment (ovary wet weight / female wet weight), number of embryos, brood size, and offspring size at birth (dry weight). Lastly, from the primary literature we added additional comparable reproductive mode and / or life history data for 16 species not in our dataset. Detailed definitions of life-history traits can be found in Table S1.

We scored poecilid species for two discrete habitat usage traits: whether a species inhabits only fresh-water (0) or, at least occasionally inhabits brackish or salt-water (1); and whether a species is found in only stagnant or slow-flowing waters (0) or, at least occasionally, inhabits fast-flowing waters (1). This information was obtained from species' habitat descriptions in books,[60–62] the public database Fishbase,[63] and primary literature. When conflicting information was found for a given species, we used the higher category. For example, if any reference indicated the species prefers, was collected in, or occasionally is found in fast-flowing water, then this was the scored category even if other references indicated the species is typically found in stagnant or slow flowing waters. This objective standard was applied so as to avoid having to make judgement calls regarding character scoring.

Lastly, we assembled data on the climatic niche of each species in our dataset following a procedure commonly used in species-distribution modeling,[64] and recently adopted in evolutionary studies.[65] We first downloaded available GPS collection coordinates from museum collection databases available through FishNet2.[66] We then compared these coordinates to the known geographic distribution of each species[60–62] and excluded coordinates that fell outside this area (likely due to data being mis-recorded or entered, species mis-identification, or species introductions). For several species that lacked museum-collection GPS-coordinate data, we estimated coordinates from written descriptions of collection localities, or obtained coordinates from primary literature sources. In total, we utilized 13,317 collection coordinates for 175 species, representing on average 76 collection localities per species. We then wrote a R script that processed the GPS coordinates, extracted matched climatic data, and calculated a single species' mean value for each of the variables. The script first removed duplicate coordinates - those less than 0.0416667 degrees, 2.5 arc-minute or ~4.5 km apart (the resolution at which we extracted climate data). We then extracted 19 bioclimatic variables from the WorldClim global climate database[22] and Google elevation database for all listed GPS collection coordinates of each species. Lastly, we calculated a single species mean value for each of the 19 bioclimatic variables (Table S1B) and elevation. We also estimated each species' geographic range size from data on FishBase.[63] This was done in two ways. First, by counting the number of occupied squares when point-occurrence data for each species is plotted on the world map using C-squares Mapper. Second, by multiplying the total number of decimal degrees between the northern and southern latitudinal range limits by the total number of decimal degrees between the eastern and western longitudinal range limits (after having first excluded any outlier point occurrence data likely due to introductions or misspecification). Furthermore, we estimated each species geographic range size in the same manner as described above but based upon the museum collection coordinates downloaded from FishNet2 and also used in the extraction of WorldClim climate data. Lastly, we obtained data on the mean, maximum, and range of altitude occurrence for each species based on values listed in museum collection records that were downloaded through FishNet2 (Table S1).

Quality control

The life history dataset contained data for 9,432 individuals, 505 populations, and 160 species gathered through dissections of museum and private collections over the past 40 years in the lab of David Reznick. Given the size and scope of the dataset, a number of checks for outliers and influential data points were enacted. We first plotted each life history trait (Length, Total wet weight, Somatic wet weight, Ovary wet weight, Female dry weight, Female lean weight, Litter, Number of embryos, Embryo stage, Embryo weight, Embryo lean weight, Reproductive tissue weight, Number of regressors, and Regressor dry weight) versus Line ID, Species code, and where appropriate, other life history variables (i.e., Length versus Total wet weight) to check for outliers and mis-entered values. Questionable values were checked for accuracy on the original data sheets, when available. Outliers that were mis-entered (for example due to a missing or mis-placed decimal point) were corrected. Eight individuals that exhibited extreme outliers for one or more life history traits (i.e., Embryo weight, Embryo lean weight, Somatic wet weight) were excluded from the calculation of all life history traits as these values are almost certainly in error, unable to be corroborated on original data sheets or likely recorded incorrectly on the original data sheet, have the potential to negatively affect the calculation of summary life-history traits, and are not excluded for other reasons such as there being less than 5 pregnant females in the population. We next plotted the calculated variables of Individual embryo weight (mg) and Individual embryo lean weight (mg) versus Line ID and Species code, having first removed stage 0 embryos. Twenty-eight embryo stages and weights were excluded from the calculation of population-level Matrotrophy Indices, mostly because such individual embryo weights were extremely small. Furthermore, when calculating the population-level Matrotrophy Indices, embryos below stage 5 were excluded to avoid unintentional inclusion of under-weight stage 0 embryos which were not fully yolked up. When calculating population-level mean life history traits all non-pregnant females were excluded. From the population-level MI and life-history dataset we calculated a single species' mean value for each trait, weighted by the number of pregnant females in each population. At this step we only included populations with five or more pregnant females; 368 populations met this criterion. When calculating the Matrotrophy Index at the species level we excluded all populations with an embryo stage range of less than 15 (in addition to excluding all populations with less than 5 pregnant females). This was done to ensure the data from which the MI was calculated was representative and trustworthy.

Preservation method

Collections were either alcohol or formalin preserved. Preservation method is known to affect the measured weights of fishes and their reproductive components. In particular, alcohol preservation tends to extract the fat from tissues, while formalin preservation does not, or does so to a much lesser extent. In general museum collections are preserved first in formalin then transferred to alcohol. Collections in the Reznick lab were generally formalin preserved with fat extracted using diethyl ether such that regular weights and lean weights (i.e., those with fat removed) were obtained. Based upon the few alcohol collections in which fat was also extracted, we know that alcohol preservation extracts most fats from the fish's body, such that the regular weights of alcohol preserved fishes are approximately comparable to the lean weights of formalin preserved fishes. We accounted for differences in preservation in the calculation of summary life history traits. We did so in two different ways. In the first, we ignore preservation method (i.e., consider its effects likely to be negligible) and calculate all life history variables using regular weights, irrespective of preservation method (and whether or not lean weights are available for formalin preserved collections). In the second method, we create composite columns for those traits in which both regular and lean weights were available (i.e., formalin preserved collections). In particular, we create a column for composite embryo weights that takes lean weights if available (formalin preserved collections) and otherwise takes regular weights (alcohol preserved collections). We do the same for female dry weight; we create a composite column for female dry weight that takes lean weight if available (formalin preserved collections) otherwise takes regular weight (alcohol preserved collections). We refer to the life history traits that take into account preservation method as 'composite' values. We then used these values (instead of embryo dry weight, and female dry weight) when calculating total embryo dry weight, total reproductive tissue dry weight, reproductive allotment dry weight for each individual, and the population-level measures of the matrotrophy index, and estimated offspring size at birth. We calculated correlation coefficients between all comparable variables ignoring and accounting for preservation method, and found them to be highly congruent in all cases ($r > 0.95$). Finally, we ran all analyses using the life history variables derived from regular weights and the composite weights (accounting for preservation method). We did not find a single difference between these two sets of analyses – indicating that the results are robust to differences in preservation method. Therefore, the life history analyses presented throughout the manuscript are those on regular weights.

Reproductive allotment

For some collections (primarily formalin preserved) we have female wet and ovary wet weights, as well as female somatic dry weight and embryo dry weights. In these collections we are able to directly calculate wet and dry reproductive allotment. For other collections (primarily museum alcohol preserved) we have female wet weights (a mix of total, somatic, or both) and embryo dry weight. In these collections we are unable to directly calculate dry reproductive allotment, since we lack female dry weights. However, ideally, we want comparable estimates of reproductive allotment despite difference in preservation method (since data from different populations is later combined into a single estimate per species, weighted by number of individuals per collection). Therefore, we implemented a procedure in which we converted total female wet weight and somatic female wet weight into estimated dry weights, such that we could calculate reproductive allotment dry weight regardless of collection type. In general, this involved fitting a regression between female wet and dry weights for all individuals with both values, and then using the parameter estimates derived from this regression to estimate an individual's somatic dry weight based upon its wet weight, for individuals that had only wet weights. Mean reproductive allotment (RA) dry mixed, was calculated as RA dry (if available) otherwise RA dry calculated. RA dry calculated was calculated as Total reproductive dry weight / Total female dry weight mixed. Total female dry weight mixed was calculated as Total female dry weight calculated (if available) otherwise Somatic female dry weight calculated + Total reproductive dry weight. Total female dry weight calculated was calculated as Total wet weight * 0.255285, and Somatic female dry weight calculated was calculated as Somatic wet weight * 0.260520. These parameter values (0.255285 and 0.260520) were determined by fitting a regression between the variables Total female dry weight ∼Total wet weight ($R^2 = 0.966$) and Female dry weight ∼Somatic wet weight ($R^2 = 0.948$), for all individuals that had wet and dry weights. We then used the parameters from these regressions to estimate the total and/or somatic dry weight of females that had only total and/or somatic wet weights. In addition, we performed the same procedure taking into account potential weight differences due to preservation method. In particular we take lean dry weights if available (formalin collections) and regular weights if lean weights are not given (primarily alcohol collections) (see above) and use these values to derive a single measure of dry reproductive allotment (RA composite dry). The only difference between the above described procedure was the parameter values relating Total composite female dry weight ∼Total wet weight and Composite female dry weight ∼Somatic wet weight were 0.215943 and 0.191772, respectively.

Finally, we ran analyses using the subset of species ($n = 69$) for which we were able to directly calculate reproductive allotment dry weight (i.e., both female dry weight and embryo dry weights directly measured) and the expanded number of species ($n = 69+60$) in which we also included species for which we estimated reproductive allotment based upon female wet weights. These analyses produced identical results (Figure S2; Table S3).

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Kansas, Museum of Natural History, Lawrence, Kansas, U.S.A.), MCP (Pontifícia Universidade Católica do Rio Grande do Sul, Museu de Ciências, Rio Grande do Sul, Porto Alegre, Brazil), MNRJ (Universidade Federal do Rio de Janeiro, Museu Nacional, Rio de Janeiro, Brazil), MZUSP (Universidade de Sao Paulo, Museu de Zoologia, Sao Paulo, Brazil), TCWC (Texas A&M University, Texas Cooperative Wildlife Collection, College Station, Texas, U.S.A.), TNHC (Texas Memorial Museum, Texas Natural History Collection, Austin, Texas, U.S.A.), TU (Tulane University, Department of Zoology, New Orleans, Louisiana, U.S.A.), UCR (Universidad de Costa Rica, Museo de Zoologia, San Jose, Costa Rica), UF (University of Florida, Florida State Museum, Gainesville, Florida, U.S.A.), UFBA (Museu de Zoologia da Universidade Federal da Bahia, Salvador, Brazil), UFRGS (Universidade Federale do Rio Grande do Sul, Departamento de Zoologia, Porto Alegre, Rio Grande do Sul, Brazil), UFRJ (Universidade Federal do Rio de Janeiro, Brazil), UIST (Universidad Industrial de Santander, Bucaramanga, Colombia), UMMZ (University of Michigan Museum of Zoology, Ann Arbor, Michigan, U.S.A.), and USNM (National Museum of Natural History, Washington D.C., U.S.A.).

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Phylogenetic tree

For our phylogenetic comparative analyses we used the timetree of Reznick et al.,[67] which contains 177 poeciliid and 116 outgroup taxa. This phylogeny was created using maximum likelihood analysis of a 28-gene (20 nuclear, 8 mitochondrial) concatenated DNA matrix.[5] Molecular dating analyses were implemented by integrating this molecular phylogeny with 16 primary fossil calibrations. Divergence times were estimated using the mcmctree program in PAML 4.4c[68] with independent rates and hard-bounded constraints.[67] The drop.tip function in the package Ape[69] was used to remove species from the timetree not present in particular analyses.

QUANTIFICATION AND STATISTICAL ANALYSIS

Phylogenetic generalized least-squares

We implemented phylogenetic generalized least-squares (PGLS) models[56,70] in the R package caper[71] to test for correlations between the matrotrophy index and life history, climate, and habitat variables, while controlling for phylogeny. Caper estimates model parameters and a measure of the magnitude of phylogenetic signal[70,72] in the model residuals (lambda, λ), using maximum likelihood. λ can range from 0, indicating no phylogenetic signal (i.e., a star phylogeny), to 1, indicating the extent of similarity between species is proportional to the amount of shared evolutionary history expected under a Brownian motion model of evolution.[70] We assess the association between each life-history trait (offspring size at birth, number of embryos, reproductive allotment), entered as the response variable, and the matrotrophy index, entered as the predictor variable, while also accounting for relevant covariates including superfetation index and standard length. Likewise, we test for an association between climatic and geographic variables (BioClim variables, elevation, and range size), entered as a response variable, and the matrotrophy index as a predictor, while also accounting for relevant covariates including superfetation index and standard length. Finally, phylogenetic logistic regression, implemented in the R package phylolm,[57] was used to evaluate the relationship between the continuous matrotrophy index and two binary habitat usage traits – inhabitation of brackish or salt-water (0/1) and inhabitation of fast-flowing water (0/1).

Our model-testing approach involved comparing a series of models designed to test our hypotheses of interest. For analyses of climate and ecology as the dependent variable, we fit three models: one which included the matrotrophy index and superfetation index, one that included only the matrotrophy index, and one that included an interaction between female standard length and the matrotrophy index. For analyses of life-history traits as the dependent variable, except for reproductive allotment, we fit the same three models but included standard length as a covariate in each model (given that life history traits are expected to positively covary with body size). Reproductive allotment already accounts for female size given that it is a ratio of female reproductive tissue weight / total female dry weight. The best model was chosen according to Akaike information criterion (AIC), defined as (-2*ln(ML likelihood))+(2*number of parameters). The best model, and that for which the output is presented, is the one with the lowest AIC, or the simplest model if the difference in AIC between alternative models is less than 2. An AIC difference of less than 2 between the best model and the alternative model indicates there is substantial support for the alternative model, a difference between 4 and 7 indicates the alternative model has considerably less support, and a difference greater than 10 indicates the alternative model is highly unlikely.[73]

We tested the effect of phylogenetic uncertainty on our results by implementing PGLS analyses using ten trees sampled at regular intervals of 50 from the maximum likelihood bootstrap analysis. Specifically, for all PGLS analyses that yielded significant results, we repeated the analysis on the best-fitting model using 10 trees from the ML bootstrap, rather than the consensus timetree. We found that conclusions did not qualitatively change (Table S3B).

BayesTraits Discrete

To investigate the joint evolution of superfetation and placentation, we employed Discrete evolution models implemented in a Bayesian framework in BayesTraits V3.[55,74] Specifically, we used Discrete independent and dependent models to evaluate the pathways by which superfetation and placentation originate and to test the hypothesis that the evolution of one of these traits promotes the evolution of the other. Discrete models require two binary traits (i.e., presence/absence of superfetation; presence/absence of placenta). For this analysis we thus converted the continuous matrotrophy and superfetation index into binary presence versus absence characters.[4] Under the independent model, the two traits evolve independently of each other and the model estimates four transition rates (the rate of gain and loss of each trait). In contrast, the dependent model estimates the transition rates between the combination of character states (presence/absence) that the two binary traits can jointly take, and thus estimates eight transition rates.[55] We first estimated the marginal likelihoods of these two alternative models (i.e., independent and dependent) in *BayesTraits* using a stepping stone sampler[53,74] with 200 stones and 200,000 iterations per stone. To identify which model fit the data better, we computed Bayes Factors (BF) as twice the difference in the logarithm of the models' marginal likelihoods. We found evidence that the dependent model fit the data better than the independent model (BF = 3.2), as BF greater than 2 is considered positive evidence for the model with the higher harmonic mean.[55] Next, we examined the posterior distributions of the 8 transition rates between the 4 possible combinations of character states (i.e., no superfetation and no placenta, no superfetation and placenta, superfetation and no placenta, superfetation and placenta) to investigate the preferential evolutionary pathway(s) from the absence to the presence of both traits.

In Discrete Independent and Dependent models, we scaled the branch lengths of the phylogeny by the default setting of 0.1, as scaling enables the algorithm to better explore parameter space when transition rates are small and difficult to estimate. We employed an exponential prior with mean seeded from a uniform hyperprior ranging from 0 to 20, and Reversible Jump (RJ). RJ can set transition rates equal to zero or to one another, thus reducing model complexity and avoiding over-parametrization.[75] MCMC chains of the Discrete Dependent and Independent models were run for 400 million iterations with a burn in of 500,000 and sampling every 200,000. MCMC analyses were run in triplicate and the three independent runs produced qualitatively similar results.

Multivariate adaptive landscape analyses

In addition to testing our *a priori* hypotheses using phylogenetic generalized least-squares (see above), we implemented adaptive landscape analyses that test for the existence of statistically differentiated multivariate phenotypic peaks or trait constellations.[43–45] The R package SURFACE[43,44] and l1ou[45] use the Ornstein-Uhlenbeck model of stabilizing selection[46,47] to first identify phenotypic shifts across the phylogeny, then collapse similar (convergent) shifts together in a backward step using an Information Criterion framework. These methods complement our PGLS analyses in that they require the same phylogenetic tree and continuous reproductive mode and life-history character data, but determine the optimal number of phenotypic peaks without having to specify any *a priori* designations of optima. We implemented these adaptive landscape analyses using six reproductive mode and life history traits – Ln (matrotrophy index), superfetation index, standard length, offspring size, offspring number, and reproductive allotment. These analyses were limited to species with data for all six traits (n = 129, the same species depicted in Figure 1). We first performed a phylogenetic principal component analysis (pPCA) on the correlation matrix of these six traits in the R package phytools[13] and retained the first 5 axes (which account for 99% of variance) in subsequent SURFACE and l1ou adaptive landscape analyses (Table S4). The pPCA was necessary because neither method accounts for covariation among multiple correlated traits (i.e., the traits are assumed to be independent) and pPCA ostensibly removes this covariation and reduces data dimensionality.[76] We implemented SURFACE using AICc (forward and backward phase), l1ou using AICc with backward test of convergence using AICc, and l1ou using pBIC and backward test of convergence using pBIC. The use of AICc as the information criterion in SURFACE often fails to select the correct model in favor of overly complex models with many changes[45,77] and a new and more conservative phylogenetic-aware information criterion (pBIC) has been proposed for model selection.[45] Thus, our comparison of different approaches (SURFACE and l1ou) and information criterion (AICc and pBIC) to determine the location of regime shifts is meant to more robustly explore the multivariate adaptive landscape. See also Figures S3 and S4 and Table S4.