

Why do placentas evolve? An evaluation of the life-history facilitation hypothesis in the fish genus *Poeciliopsis*

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Summary

1. The Northern Clade of the fish genus *Poeciliopsis* includes six closely related species, three of which lack placentas and three that have placentas but vary in the extent of post-fertilization provisioning.
2. We used this diversity to evaluate a series of hypotheses proposed in earlier publications concerning why the placenta has evolved. All hypotheses share the attribute of arguing that the placenta evolved to enhance the evolution of some other life-history trait, such as to reduce the age at maturation or to increase offspring size. We refer to these hypotheses collectively as ‘life-history facilitation hypotheses’.
3. A general way to evaluate the plausibility of such proposals is to ask whether the evolution of the placenta is predictably associated with the evolution of other components of the life history.
4. We evaluated such associations in two ways. First, we performed a multivariate analysis of life-history data for fish collected and preserved in nature. This analysis included 16 populations across all six species. Secondly, we performed a more complete quantification of the life histories of the laboratory descendents from five populations representing four species, then performed a similar multivariate analysis. The laboratory study added information about the timing of reproduction (age at maturity, frequency of reproduction).
5. Both analyses yielded similar results, which were that the evolution of increased placentation is correlated with the evolution of a smaller size at first reproduction, the production of fewer and smaller offspring per litter, but an increase in the number of litters that were developing simultaneously in the ovary (superfetation). Increased placentation is associated with progressively earlier ages at maturation and shorter intervals between the birth of successive litters of offspring. Overall, increased placentation was associated with an increase in the rate of production of offspring early in life. A peculiar attribute of placentation is that this increase in the rate of offspring production can be attained despite a simultaneous reduction in the proportional quantity of resources devoted to reproduction.
6. These trends support one of the life history facilitation hypotheses, which is that placentation facilitates earlier maturity and a higher rate of reproduction early in life. They also suggest a possible connection between the evolution of the placenta and the well-established theory of life-history evolution, since these same life history attributes are predicted to evolve in response to exposure to high extrinsic rates of adult mortality.

Key-words: adaptation, life-history evolution, maternal effect, phenotypic plasticity, predation, reproductive strategies, placenta, placentation

Introduction

The ability of mothers to provide nourishment to developing embryos by a source other than yolk (matrotrophy) is

not an exclusive trait of the mammalia; it is widespread across animal taxa (e.g. terrestrial and aquatic gastropods, Baur 1994; Von Rintelen & Glaubrecht 2005; clams, Kornushin & Glaubrecht 2003; pseudoscorpions, Makioka 1968; flies, Meier, Kotrba & Ferrar 1999; cockroaches, Williford, Stay & Bhattacharya 2004; isopods, Warburg &

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Rosenberg 1996; several fish groups, Wourms, Grove & Lombardi 1988; amphibians, Greven 1998; Wake 1993; among others). Organisms exhibiting this resource allocation strategy are believed to be subject to unique biological consequences when compared with organisms that are viviparous but only allocate resources in the form of yolk to feed developing young (lecithotrophy). For example, the evolution of matrotrophic reproduction is expected to alter the dynamics of life-history trade-offs (Trexler & DeAngelis 2003) and costs and benefits of parental care (Smith & Wootton 1995; Reynolds, Goodwin & Freckleton 2002).

Placental reproduction is a specialized form of matrotrophy. The placenta is defined as an apposition of maternal and embryonic tissue that provides for the nourishment of the developing embryo and sustains its metabolic needs (respiration and excretion) throughout development (Mossman 1937). Placental matrotrophy is hypothesized to intensify maternal effects because of the prolonged and intimate contact between the mother and developing young (Trexler 1985; Korsgaard & Weber 1989; Lombardi 1996; Paulesu, Romagnoli & Bigliardi 2005). Moreover, the evolution of placental reproduction may open a new arena for parent-offspring conflict (Haig 1993; Crespi & Semeniuk 2004). Besides mammals, placental organisms include scorpions (Farley 1998), onychophorans (Huebner & Lococo 1994), bivalves (Korniushin & Glaubrecht 2003), bryozoans (Woolacott & Zimmer 1975), squamate reptiles (Flemming & Blackburn 2003), and chondrichthyan and osteichthyan fishes (Wourms, Grove & Lombardi 1988; Reynolds, Goodwin & Freckleton 2002).

Studies of life-history evolution address how traits like the age at maturity, reproductive effort or offspring size may evolve in response to features of the environment, but have done little to explore matrotrophic reproduction as a life-history trait, as evidenced by the absence of any discussion of this aspect of the life history in the most recent comprehensive reviews (Roff 1992, 2002; Stearns 1992). Trexler & DeAngelis (2003) simulated the fitness costs and benefits of matrotrophy and found that matrotrophy is more likely to evolve in environments where food availability is constantly high – a prediction that is consistent with some earlier proposals (Thibault & Schultz 1978) and empirical results (Reznick, Callahan & Llauredo 1996; Trexler 1997; Swain & Jones 2000; Marsh-Matthews & Deaton 2006; Pires, McBride & Reznick 2007).

Some authors have proposed a possible bridge between the evolution of matrotrophy and the evolution of other life-history attributes by suggesting that the evolution of matrotrophy enhances the evolution of other life-history traits. For example, matrotrophy has been proposed to facilitate the evolution of larger litter size, larger offspring size at birth, earlier maturity or increased survivorship early in life (Thibault & Schultz 1978; Blackburn, Vitt & Beuchat 1984; Wourms & Lombardi 1992; Wourms 1993; Trexler 1997; Holbrook & Schal 2004; Schrader & Travis 2005; Wildman *et al.* 2006). We refer to these hypotheses collectively as 'life-history facilitation hypotheses' because they share the attribute that the

placenta is seen as facilitating the evolution of some other feature of the life history.

Here, we evaluate the plausibility of life-history facilitation hypotheses by exploiting natural variation among closely related species in the genus *Poeciliopsis* in the extent of placental provisioning (Reznick, Mateos & Springer 2002). We ask whether the evolution of increased placental provisioning is correlated with the evolution of other features of the life history. If the evolution of placentotrophy is predictably associated with the evolution of other life-history traits, such as age at maturity or reproductive allocation (RA), then it may be possible to support one of the existing hypotheses for the evolution of placentas. In addition, it may be possible to bridge proposed hypotheses for the evolution of placentas with the existing, well-developed hypothetico-deductive framework for life-history evolution. For example, high adult mortality was predicted and has been shown empirically to select for earlier age at maturity, increased reproductive effort and increased early-life fecundity (Charlesworth 1980; Reznick, Bryga & Endler 1990; Clobert, Garland & Barbault 1998; Gasser *et al.* 2000). Some authors have independently proposed that matrotrophic reproduction facilitates the evolution of these same traits (Blackburn, Vitt & Beuchat 1984; Wourms 1993; Trexler 1997). If such consistent correlations between placentation and other features of the life history exist, then we could hypothesize, for example, that placentotrophy facilitates the adaptive response of organisms subject to high adult mortality.

The Northern Clade of *Poeciliopsis* has the requisite variation in maternal provisioning for us to evaluate these hypotheses. This clade is found primarily north of the Trans-Mexican Volcanic Belt (Mateos, Sanjur & Vrijenhoek 2002; Reznick, Mateos & Springer 2002; Mateos 2005). It contains six species, three of which have little or no post-fertilization maternal provisioning (Fig. 1: *Poeciliopsis monacha*, *Poeciliopsis viriosa*, *Poeciliopsis infans*). The remaining three species vary from having sufficient post-fertilization provisioning to sustain a 31% increase in offspring dry mass between fertilization and birth (*Poeciliopsis occidentalis* – Constantz 1980),

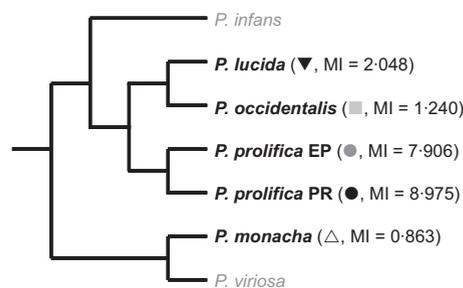


Fig. 1. Phylogenetic relationships of the Northern Clade of the genus *Poeciliopsis* (Mateos, Sanjur & Vrijenhoek 2002). Phylogeny is shown with Pagel's (1992) arbitrary branch lengths, as used in our phylogenetically based analyses. Taxa used in laboratory study are shown in black font, including two populations of *Poeciliopsis prolifica*. In parentheses, their respective symbols used throughout the manuscript and their estimated matrotrophy index (MI; see text and Table 1).

to more than doubling offspring dry mass during development (*Poeciliopsis lucida* – Thibault & Schultz 1978) to having a greater than sevenfold increase in dry mass between fertilization and birth (*Poeciliopsis prolifica* – Pires, McBride & Reznick 2007). This diversity among close relatives affords us the opportunity to evaluate whether there is correlated diversity in the remainder of the life history.

We first compare the life histories of laboratory-reared individuals from four species (five populations) raised under common-garden conditions. We then compare life-history traits of wild-caught and preserved fish from 16 populations that represent all six species in the clade (Fig. 1). In both cases, we ask: (i) Is the evolution of increased placentation correlated with other features of the life history? (ii) If so, then do these correlations offer us clues to its value as a life history adaptation? Our comparisons of wild-caught fish enabled us to sample populations from all six species in the Northern Clade and to represent each species with two to five populations, thus providing broad coverage of the clade and large sample sizes. The limitations of such data are that we are not being able to estimate all life-history traits of interest and cannot know the extent to which the observed life-history variation is dictated by environmental or genetic effects (Garland & Adolph 1991). Our laboratory study only included four species and smaller sample sizes, but yields greater confidence that the differences among species have a genetic basis in addition to providing a more complete description of the life history. One key question is whether these two forms of comparison yield similar patterns and hence support the generality of our results.

Materials and methods

THE GENUS *POECILIOPSIS*

The genus *Poeciliopsis* (Cyprinodontiformes: Poeciliidae) is composed of 20 extant, viviparous species, distributed mostly in Pacific slope drainages from southern Arizona to western Colombia (Mateos, Sanjur & Vrijenhoek 2002). The placental species in this genus transfer resources from the mother to developing young through the follicular placenta, which is formed by a highly modified, vascularized epithelium of the follicular sac (the maternal membrane) in close contact with a vascularized pericardial sac (the embryonic membrane) or expanded yolk sac; embryonic body and fin epithelia may also be involved in resource acquisition (Turner 1939, 1940, 1947; Grove & Wourms 1991, 1994; Wourms & Lombardi 1992). All species in this genus also have superfetation, which is the ability to simultaneously carry multiple litters of embryos at different stages of development (Turner 1937, 1947; Scrimshaw 1944). Species vary from having 2 to 5 simultaneous litters of embryos developing in their ovary (Thibault & Schultz 1978).

SPECIES AND COLLECTION SITES

All six species were represented in our field collections. We either made the collections ourselves or dissected subsets of collections that had been archived in museums (See Appendix S1 for collection details). Four out of the six species in this clade are represented in the

laboratory study. The founders of the *P. monacha* laboratory stock were collected in 1999 at the Jaguari stream, Fuerte drainage, Sonora State. *Poeciliopsis occidentalis* livestock was collected in March 1976 at Altar River near Oquitoa, Sonora State, *P. lucida* livestock was collected in May 2003 at a hot springs in the Fuerte River drainage, c. 50 km east of the village of Güirocoba, Sonora State. Two populations of *P. prolifica* were collected in January, 2004 from El Palillo River, between San Blas and Tepic, Nayarit State and from Presidio River, near Mazatlán, Sinaloa State. In all analyses of laboratory data, we treat each population of *P. prolifica* separately. Significant life history differences between these two populations have been shown to have a genetic basis (Pires, McBride & Reznick 2007).

DISSECTIONS OF WILD-CAUGHT FISH

We first measured female standard length (tip of lower jaw to the outer edge of the hypleural plate) and wet weight. The ovary was then removed through a mid-ventral incision, then the female and ovary were weighed separately. Because all species of *Poeciliopsis* have superfetation, the ovary often contained multiple litters of young in different stages of development. The number of young in each litter, their stage of development and their wet weight were recorded.

We staged embryos with the compressed developmental series adopted for staging embryos of lecithotrophic species (Reznick 1981; Reznick & Endler 1982) as modified by Haynes (Haynes 1995) for characterizing development in matrotrophic species. Our broad categories were 'no development-blastodisc' (stages 1–6), 'uneyed' (stages 7–12), 'early eyed' (stages 13–17), 'mid-eyed' (stages 18–20), 'late-eyed' (stages 21–24) and 'very late eyed' (stages 24–25), which roughly correspond to the numbered stages in parentheses used by Tavolga & Rugh (1947) to describe embryonic development in *Xiphophorus maculatus*, another member of the family Poeciliidae. Embryos that were in the same stage of development were grouped together as a litter, then dried at 55 °C overnight and stored in a desiccator until they were weighed to the nearest 0.01 mg on a Mettler AE163 electrobalance.

The degree of placentotrophy was estimated with the Matrotrophy Index (MI; Reznick, Mateos & Springer 2002) for each individual. MI equals the average offspring dry mass at birth divided by the average dry mass of fully provisioned ova. We estimated MI in the same fashion in fish from the museum collections and laboratory study. Similar approaches have been used to quantify the degree of placentotrophy in snakes, lizards and other fish species (e.g. Wourms, Grove & Lombardi 1988; Stewart & Thompson 2003; Thompson & Speake 2006).

The life history of each population was characterized with the minimum and mean size of reproducing females, ovary wet mass relative to body size, the degree of superfetation and MI, the number of developing litters, the number of offspring per litter, their stage of development, mean dry offspring weight and reproductive allotment (RA). Minimum size at first reproduction has served well as an index of the age and size at maturity in guppies (Reznick & Endler 1982). RA equals the percent of total dry mass that consists of developing offspring. In multivariate analyses that included body size, we substituted total ovary mass for RA as a measure of reproductive investment.

LABORATORY PROCEDURES

We reared laboratory-born (second generation or beyond) fish under controlled conditions in the vivarium at the University of California,

Riverside to minimize environmental effects that may influence life-history comparisons (Trexler 1989; Garland & Adolph 1991). All procedures were approved by the UCR Institutional Animal Care and Use Committee.

The natural populations of the Northern Clade have geographic ranges that span the deserts and seasonal dry forests of southern Arizona and the Pacific slopes of northern Mexico. Water temperature and quality fluctuates widely as a function of season and rainfall. One population of *P. occidentalis* from Arizona is known to cease reproduction during the winter (Constantz 1979), but all of the preserved collections that we worked with contained at least some gravid females, regardless of when they were collected, which also means regardless of environmental conditions. Most species thus appear to breed throughout the year. We used water quality in our laboratory studies that matched conditions observed in most native streams for at least a portion of the season when the fish would be reproducing – pH of 7.8–8 and total hardness of 250–300 p.p.m. Laboratory water temperatures ranged from 25 to 28 °C.

Wild-caught adult females of *P. lucida* and *P. prolifica* were kept in isolation until they produced a litter of young that was then assigned a distinct pedigree and reared to maturity. Male and female offspring were isolated before maturity. Crosses were then made among different pedigrees in a breeding design that maximized the retention of the genetic variation present in the wild-caught stocks in the F₂ generation, as reported elsewhere (Pires, McBride & Reznick 2007). *Poeciliopsis monacha* and *P. occidentalis* were derived from older laboratory stocks, but were submitted to the same procedures as individuals from other taxa. Offspring from the F₂ generation were fed *ad libitum* until they were 25 days-old. We then isolated four individuals from each litter in 8 L tanks distributed in randomized blocks. Because it was not possible to determine the sex of the offspring at the time of isolation, it was not possible to equally represent males and females.

We randomly assigned two individuals from each F₂ litter to one of two levels of food availability. Individuals received food twice daily consisting of liver paste (morning) and *Artemia* nauplii (afternoon; details in Appendix 2 of Reznick 1980). Food rations were determined after observation of growth and reproduction of different *Poeciliopsis* species in a pilot study (Appendix S2). The 'high-food' treatment ranged from twice the level of the 'low-food' treatment at the start of the experiment to about three times the level of the 'low-food' treatment as females aged and grew (Appendix S2). The differential between high and low food increased overtime to accommodate the increasing differences in size and presumably basal metabolic demands. We measured standard length (mm, using a digital caliper) and mass (in mg, with an electrobalance) of all individuals every 2 weeks. Fish were anaesthetized in a buffered solution of MS-222 before they were measured.

A Mature F₁ male was added for 24 hours to the tanks with unrelated females 2 weeks after female isolation and every other week thereafter. Males were taken from a large stock and returned to the stock after mating, so a female likely encountered many different males over the course of the study. Food rations were increased by 5 µL when a male were present.

The age and size at maturity in F₂ males were determined on the basis of the metamorphosis of the anal fin (Turner 1941). Female age at first parturition was used as a proxy for female age at maturity. All females were kept in the experiment for 8 weeks after the age at first parturition. During this time, we quantified the frequency of reproduction (interlitter interval, the number of days between the birth of consecutive litters), litter size (number of embryos born in a single litter) and mean offspring dry mass at birth per litter. All

offspring and mothers were euthanized with an overdose of MS-222 prior to preservation. Newborn offspring were preserved in 5% formalin on the day of birth. We preserved all females in 5% formalin after 8 weeks and dissected them to further characterize their life histories, following the same protocol as for the wild-caught fish. The dependent variables associated with each individual were thus the same as those for the field collections, plus the individual's age at first parturition, interlitter interval, litter size and offspring mass at birth.

STATISTICAL ANALYSES

In our laboratory study, we used two-way univariate analyses of variance and covariance. The two main effects were taxa and food availability. We evaluated whether or not female wet mass at first parturition was an appropriate covariate for female life-history traits, except when testing the effects of traits that included mass in the estimation of the trait value (e.g. RA). Female wet mass was included in the analysis when the assumptions of the analysis of covariance were satisfied. We ran separate analyses for males and female age and size at maturation. Values for all variables were natural log-transformed if this normalized the distribution of the residuals, which was the case for all traits except superfetation and average interlitter interval.

We evaluated the correlations between average taxa values for MI and other female life-history traits using both conventional (i.e. assuming a 'star phylogeny', or that all taxa radiate from a single common ancestor) and phylogenetically based statistical analyses [phylogenetic generalized least-squares (PGLS) model, Matlab program MECorrPHYSIG.m (GLS Estimating Method) from Ives, Midford & Garland (2007)]. For the latter, we used the most current evolutionary hypothesis for the relationship among *Poeciliopsis* species (Mateos, Sanjur & Vrijenhoek 2002; Fig. 1). We used a tree with arbitrary branch lengths (Pagel 1992) in all phylogenetically based statistics because we do not know the real divergence time between species (Fig. 1). We performed similar correlation analyses on the data derived from wild-caught individuals. Here we used Pagel's arbitrary branch lengths (Pagel 1992) at the species level. As we do not know the phylogenetic relationship between populations within a species, we included populations as soft polytomies with branch lengths of 0.5. To judge whether the conventional or phylogenetic model better fit the data, we compared the likelihood of the PGLS and the 'star' models. We used $\alpha = 0.01$ to determine statistical significance of bivariate correlations to reduce the risk of Type I error due to multiple comparisons.

We further explored how combined life-history traits separated individuals and taxa in multivariate analyses that excluded MI, ova dry mass and RA. MI was excluded because it was used as a dependent variable when interpreting the effect of the composite variable obtained in the multivariate analyses. Ova dry mass and RA were excluded because they were known *a priori* to be correlated with MI and female wet mass at first parturition, respectively, because of the way MI and RA are calculated (see above). Ovary dry mass, an estimate of reproductive investment that is used in the calculation of RA, was used instead of RA in multivariate analyses.

We evaluated the probability of correct classification of laboratory-reared individuals to their taxon in a discriminant function analysis that grouped individuals based on their composite life histories. Due to high multicollinearity (see Results), the relative importance of each trait in each discriminant function coefficient were not readily interpretable. We instead based our interpretations on a Principal Components Analysis (PCA). We first obtained a

factor score per laboratory-reared taxon from the first component of a PCA on taxon mean values. We then correlated this composite life-history variable with the respective value for the MI for each taxon to evaluate which composite changes in life-history correlate with an increase in placentotrophy. These scores and the MI were analysed as a star phylogeny because the star phylogeny produced a better fit to the data, as indicated by the higher likelihood of the model.

We performed a similar PCA analysis on the field data, but with a reduced suite of variables. Each population was represented by a single value, so the population was the level of replication. This approach differed from our analyses of the laboratory data, in which the individual was the level of replication. The life-history traits for the field analyses included minimum size of reproductive females (a field surrogate for the size at first parturition), the mean level of superfetation, the mean number of offspring per litter, the projected mass of offspring at birth (derived from a regression that describes the relationship between stage of development and offspring mass) and the mean total reproductive mass (the dry mass of all developing embryos and ovarian tissues). The PCA was conducted in a conventional, non-phylogenetic fashion (i.e. assuming a star phylogeny). The PCA scores and MI values for each population were then analysed by PGLS to obtain correlations between MI and each of the PCA axis because the bivariate correlations showed that the analysis that included phylogeny provided a better fit for the field data.

Results

LIFE-HISTORY UNIVARIATE COMPARISONS AMONG TAXA – LABORATORY DATA

Food ration was often significant as a main effect, but never interacted with taxon. We have simplified the presentation of the results reported here by just reporting on the main effect of taxon; details of the effects of food appear in Appendix S2.

Taxa differ significantly in all male and female life-history traits measured (Table 1). MI values are rank ordered as in our earlier study (Reznick, Mateos & Springer 2002): *Poeciliopsis monacha* embryos lose dry mass during development, thus generating an MI value of less than 1 (MI = 0.86). *Poeciliopsis occidentalis* (MI = 1.25) and *P. lucida* (MI = 2.05) gain small to moderate amounts of dry mass during development, while *P. prolifica* embryos increase in mass eight- to ninefold during development (MI = 7.91 and 8.98). The age and size at maturity in males and at first parturition in females ranks in the reverse order of MI: *Poeciliopsis prolifica* are the smallest and youngest at maturity while *P. monacha* are the oldest and largest at maturity. Most other features of the life history also rank-order with MI; the degree of superfetation is lowest for *P. monacha* (1.3 litters) and largest for *P. prolifica* (2.8 and 3.1 litters), interlitter interval is longest for *P. monacha* (13.4 days) and shortest for *P. prolifica* (6.9 and 7.7 days), and RA is largest for *P. monacha* (0.25) and smallest for *P. prolifica* (0.10, 0.08). The two species with moderate levels of matrotrophy always rank between these two extremes. The only feature of the life history that is not well associated with MI is litter size.

The rank orderings of ova size at fertilization vs. embryo size at birth reveal how maternal provisioning changes with the evolution of the placenta in this clade. The average dry mass of the mature ova is largest in *P. monacha* (2.46 mg) and smallest in *P. prolifica* (0.11 and 0.12 mg). While the rank ordering for the dry mass of offspring at birth is the same, the magnitude of the differences among species is compressed (*P. monacha* = 1.97 mg, *P. prolifica* = 0.91 and 0.98 mg). *Poeciliopsis occidentalis* and *P. lucida* are intermediate between these two species both for ova weight and offspring weight at birth. The progressively higher MI values in *P. occidentalis*, *P. lucida* and *P. prolifica* are thus

Table 1. Untransformed grand means across food treatments and standard error (in parentheses) of life-history traits for laboratory-reared *Poeciliopsis* species. Either untransformed or natural log-transformed data were used for analyses, depending on distribution deviation from normality and homogeneity of variances. Sample size varied depending on presence of outliers and availability of data per female. The matrotrophy index (MI) for each taxon is not exactly the ratio between mean offspring dry mass and mean ova dry mass because the MI value presented on the table was calculated with data from only those females for which both ova and offspring dry mass were available, whereas the mean value reported for ova and offspring dry mass incorporated all available data for these traits across all females

Variable	<i>Poeciliopsis monacha</i>	<i>Poeciliopsis occidentalis</i>	<i>Poeciliopsis lucida</i>	<i>Poeciliopsis prolifica</i> EP	<i>Poeciliopsis prolifica</i> PR	F-ratio (d.f.)
Number of females	17	6	12	23	22	
Matrotrophic index	0.86 (0.10)	1.24 (0.13)	2.05 (0.17)	7.91 (0.45)	8.98 (0.65)	164.39 (4.55)**
Female age at first parturition (days)	147 (8)	148 (13)	119 (5)	105 (4)	93 (3)	17.39 (4.70)**
Female wet mass at first parturition (gm)	0.48 (0.05)	0.39 (0.07)	0.38 (0.05)	0.29 (0.03)	0.26 (0.02)	14.96 (4.70)**
Ova dry mass (mg)	2.46 (0.17)	1.67 (0.08)	0.78 (0.06)	0.11 (0.01)	0.12 (0.01)	431.83 (4.62)**
Offspring dry mass at birth (mg)	1.97 (0.12)	1.91 (0.07)	1.35 (0.03)	0.91 (0.03)	0.98 (0.03)	34.85 (4.69)**
Litter size	3.1 (0.5)	2.2 (0.7)	3.1 (0.5)	2.5 (0.4)	1.9 (0.3)	2.77 (4.70)*
Ovary dry mass (gm)	0.037 (0.005)	0.011 (0.005)	0.016 (0.002)	0.009 (0.001)	0.008 (0.001)	24.56 (4.69)**
Superfetation	1.3 (0.1)	2 (0.3)	2.2 (0.1)	3.1 (0.1)	2.8 (0.1)	40.71 (4.70)**
Average interlitter interval (days)	13.4 (0.6)	11.8 (1.0)	11.4 (0.3)	6.9 (0.3)	7.7 (0.4)	36.30 (4.61)**
Reproductive allocation	0.25 (0.01)	0.11 (0.02)	0.16 (0.01)	0.10 (0.01)	0.08 (0.01)	36.19 (4.70)**
Male age at maturity (days)	102.4 (13.3)	91.7 (5.7)	80.6 (7.5)	63.2 (2.9)	66.8 (3.7)	6.72 (4.50)**
Male wet mass at maturity (gm)	0.18 (0.03)	0.16 (0.01)	0.12 (0.01)	0.05 (0.00)	0.06 (0.00)	38.65 (4.51)**

* $P < 0.05$ (two-tailed); ** $P < 0.01$.

attributable to a proportionally greater decrease in the size of the egg at fertilization rather than to an increase in offspring size at birth.

CORRELATES OF INCREASED PLACENTOTROPHY – LABORATORY RESULTS

When comparing taxa with conventional statistical analyses (i.e. assuming no hierarchical relationship among taxa), most taxon means for life-history traits are highly correlated with each other with $r > 0.9$ (Table 2a). Mean values for the MI showed a high positive correlation with the degree of superfetation and a high negative correlation with age at first parturition, wet mass at first parturition, offspring size at birth and interlitter interval (Table 2a). MI is also negatively correlated with RA and ovary dry mass, but not as strongly so as with other traits (Table 2a). Ovary dry mass and RA are highly correlated with each other (Appendix S3) despite differences in body size across taxa.

Incorporating phylogenetic information (PGLS model) causes a slight overall reduction in the magnitude of the correlation coefficients involving the MI, which remained high nonetheless (Table 2a, Fig. 2). MI is only significantly correlated at $P < 0.01$ with interlitter interval and ova dry mass, the latter correlation being in part an artefact of the way MI is calculated.

When discriminant function scores are used to verify how useful life-history traits are to separate individuals among taxa, 84.3% of individuals are classified within their correct population/species. The only misclassifications occur for 11 individuals between the two *P. prolifica* populations, so 100% of individuals are correctly classified to species.

The first component of the principal component analysis on taxon means for female life-history traits accounts for 81.9% of the total variance (Table 3a) and defines an axis that mainly separated species with a high degree of superfetation, low offspring dry mass at birth, low age and size at first parturition, short interlitter interval and low ovary dry mass from those with the opposite trends in their life history (Table 3a). Litter size, the only life-history trait that is not highly correlated with any other trait, accounts for most of the variance explained in the second component. There is a strong inverse relationship between the MI value per taxon and the taxon factor score for the first principal component ($r = -0.978$, d.f. = 3, $P = 0.004$; Fig. 2a). We repeated this analysis twice, each time including only one of the two *P. prolifica* populations. In both cases the Spearman Rank Order correlation has a value of -1.0 (d.f. = 2, $P < 0.01$). The Pearson product-moment correlation has values of -0.969 (d.f. = 2, $P = 0.031$) and -0.965 (d.f. = 2, $P = 0.035$); the trends are clearly the same using either population. Figure 2a shows that the value of MI is inversely related to the first principal component. This means that an increase in post-fertilization maternal provisioning (higher value of MI) is correlated with a progressive increase in the degree of superfetation, shorter interlitter interval, earlier age at first parturition, smaller offspring and a smaller total RA. Differences among species

in MI are thus well correlated with the evolution of other features of the life history.

FIELD COLLECTIONS

The life-history traits that characterize each collection (Table 4) were used as a basis for first performing bivariate correlations between MI and the individual life-history traits. Our correlation analyses (Table 2b) reveals a pattern qualitatively similar to the laboratory data. MI is negatively correlated with the smallest size and average size of reproducing female, litter size, size of offspring at birth, RA and ovary mass, but positively correlated with superfetation. Three of these correlations are significant assuming a star phylogeny (smallest size at first parturition, maximum number of litters per female, superfetation), but only superfetation is significant in correlations incorporating phylogeny. The results of the PCA are again qualitatively similar to the laboratory studies, but with much weaker discrimination among species and a weaker association with MI. The factor loadings for the first PC, which accounted for 49.9% of the variation, have a negative contribution from superfetation but positive contributions from the minimum size of reproductive females (a surrogate for size and age at maturity), litter size, offspring size at birth and the ovary dry mass, with ovary dry mass being the most important source of discrimination among species (Table 3b). The correlation between the value of MI and the PCA scores for each of the 16 populations reveals a significant negative correlation between MI and the first principal component ($r = -0.725$, d.f. = 14, $P = 0.001$; Fig. 2b). The one feature of the field data that represents a departure from the laboratory data is the result for offspring size in *P. infans*. This species has low values for MI (0.65–0.99) but also consistently low values for offspring mass at birth (0.64–0.85 mg). This offspring dry mass at birth is comparable with *P. prolifica* but much smaller than seen in the other species with little or no post-fertilization maternal provisioning (*P. monacha*, *P. viriosa*). In the laboratory data, the species with low MI values produce larger offspring. Offspring size evolution is thus not strictly linked to the evolution of placentation.

Discussion

We found a significant association between MI (an index of the pattern of maternal provisioning) and other aspects of the life history in both laboratory and field data. In both data sets, we found that increased post-fertilization maternal provisioning (higher MI values) was associated with an increase in the degree of superfetation and a decrease in offspring size and RA. Our laboratory study also showed that increased post-fertilization provisioning was associated with earlier maturity and a smaller size at maturity. The similarity of the results in our laboratory study of four species (five populations) and wild-caught fish from all six species (16 populations) in the Northern Clade supports the generality of these associations between maternal provisioning and other components of the life history. These similarities between labora-

Table 2. Correlation coefficients between the matrotrophy index and other life-history traits for laboratory-reared and wild-caught fish. Natural log of the likelihood value for each correlation shown in parenthesis. Values reported from analyses with untransformed taxa means ('Star') and taxa means corrected for phylogenetic effects ('PGLS'). The correlation coefficients significant at $P < 0.01$ [two-tailed, critical value = 0.959 for laboratory data (3 d.f.) and 0.623 for field data (14 d.f.)] are shown in bold. We used a conservative significance level to reduce the risk of Type I error due to multiple comparisons. See Appendix S3 for the full correlation coefficient matrices for all life-history variables. All variables but superfetation and interlitter interval were natural log-transformed

a. Laboratory data									
Phylogeny	Age at first parturition	Wet mass at first parturition	Ova dry mass	Offspring dry mass	Litter size	Superfetation	Interlitter interval	Ovary dry mass	Reproductive allocation
Star	-0.959 (-1.035)	-0.976 (-1.46)	-0.998 (-1.594)	-0.974 (-0.850)	-0.551 (-4.990)	0.953 (-5.700)	-0.984 (-9.964)	-0.733 (-9.033)	-0.756 (-7.447)
PGLS	-0.913 (-0.28)	-0.943 (-0.112)	-0.994 (-2.987)	-0.946 (-1.416)	-0.531 (-5.081)	0.894 (-7.027)	-0.963 (-10.967)	-0.626 (-10.392)	-0.652 (-8.726)
b. Field data									
Phylogeny	Smallest size at first parturition	Average female size at first parturition	Maximum number of litters in a female	Superfetation	Fecundity	Litter size	Estimated dry weight of offspring at birth	Reproductive allocation	Dry weight of reproductive tissue
Star	-0.704 (-9.668)	-0.599 (-9.789)	0.786 (-12.836)	0.885 (-14.731)	0.019 (-34.174)	-0.538 (-31.688)	-0.349 (-27.054)	-0.526 (-27.773)	-0.684 (-35.233)
PGLS	-0.311 (-5.206)	-0.493 (-2.245)	0.468 (-2.951)	0.629 (-5.254)	-0.178 (-27.460)	-0.409 (-24.978)	-0.349 (-13.846)	-0.281 (-20.654)	-0.53 (-27.566)

Description of variables: Dry weight of reproductive tissue = average dry weight of all eggs and embryos found in reproductive females; Reproductive allocation = [dry mass of embryos/(dry mass of embryos + dry mass of female)], from field collections preserved in formalin or [dry mass of embryos/wet mass of alcohol-preserved females]*3.592, for females preserved in alcohol; Estimated dry weight of offspring at birth = estimated dry weight at stage 45 based on parameters of regression model between stage of development and embryonic dry weight; litter size = litter size of the average-sized female (the average number of young per litter, estimated from applying the regression of litter size on female size to the average female length); Superfetation = average number of litters per female at the time of dissection; Maximum number of litters per female = maximum number of litters found in a female.

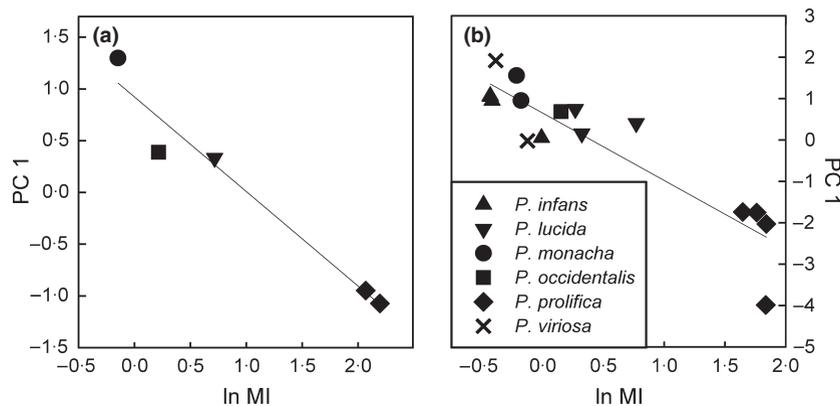


Fig. 2 Relationships between matrotrophy index (MI) and first principle component (PC) for (a) laboratory and (b) field samples.

Table 3. Component matrix for the Principal Component Analysis (non-phylogenetic) of taxon means for laboratory-reared and wild-caught female life-history variables. Factor loadings, eigenvalues and percentage of variance explained for the first three components are shown

a. Laboratory data

Variable	Component		
	1	2	3
Age at first parturition	0.902	-0.345	0.193
Female wet mass at first parturition	0.998	0.019	0.023
Offspring dry mass	0.923	-0.381	0.034
Litter size	0.661	0.705	0.253
Superfetation	-0.973	0.095	0.195
Interlitter Interval	0.981	-0.102	0.02
Ovary dry mass	0.853	0.434	-0.266
Eigenvalue	5.733	0.968	0.212
Percentage of total variance explained	81.90%	13.80%	3.00%

b. Field data

Variable	Component		
	1	2	3
Smallest size of reproductive females	0.725	-0.255	-0.467
Superfetation	-0.68	0.459	0.382
Litter size	0.72	-0.249	0.638
Estimated offspring size at birth	0.46	0.834	-0.285
Ovary dry mass	0.883	0.331	0.306
Eigenvalue	2.498	1.143	0.946
Percentage of total variance explained	49.90%	22.80%	18.90%

tory and field results are present in spite of the laboratory fish being reared together in a controlled environment while the field populations were each collected from their own habitat. The consistency of these results enables us to infer that they represent properties of the species under study, rather than artefacts created by the choice of environment for the laboratory study. Since the laboratory data were derived from fish reared for multiple generations in a common environment, we can also infer that the differences in life histories are likely to have a genetic basis.

Our results are consistent with those of Thibault & Schultz (1978), who analysed the life histories of three of the four species included in our laboratory study (*P. monacha*, *P. lucida*, *P. prolifica*). Most of their data were derived from wild-caught specimens and some of the reported traits were not amenable to quantitative analyses. For example, their reported estimates of the age at maturity were the earliest observed age at first parturition in fish reared in tanks with siblings and on uncontrolled levels of food availability. In spite of the differences in methods, they reported the same qualitative differences among these species for the degree of superfetation, interlitter interval, litter size and offspring size at birth. Their results thus reinforce the repeatability of the life history differences among these species.

A key result in our laboratory study is the correlation between the MI and factor scores for the first principal component, a composite index of the life history (Table 3; Fig. 2). This association shows that the evolution of increased post-fertilization maternal provisioning in this clade is strongly correlated with the evolution of increased superfetation and decreased interlitter interval, age at maturity, offspring size and, to a lesser extent, RA. Number of offspring in the first litter, although significantly different among taxa, is not predicted by the MI. The one aspect of this correlated complex of life-history traits that is changed with the inclusion of two additional species in the field data is offspring size, as *P. infans* produces comparatively small offspring, but otherwise shares similar life-history attributes to the other species with low MI values.

The net effect of the set of life-history traits summarized in our univariate and multivariate analyses of laboratory data is a higher rate of offspring production early in life in species with high MI values. This difference can be visualized with calculations of the cumulative expected number of young that will be produced by each taxon, based upon the mean values for age at first parturition, frequency of reproduction and number of offspring per litter (Fig. 3). An important feature of these comparisons is that the fecundity advantage of *P. prolifica* is not achieved because they produce larger litters than *P. monacha* (in fact, they produced smaller litters on average), but rather because they begin to reproduce at an

Table 4. Summary of life-history traits for wild-caught *Poeciliopsis* individuals, showing means per taxon with standard error (in parenthesis). All data reported here are untransformed. Either untransformed or natural log-transformed data were used in analyses, depending on distribution deviation from normality and homogeneity of variances. Sample size varied depending on presence of outliers and availability of data per female

Species	Matrotrophy Index	Average female size (mm)	Superfetation	Litter size	Estimated ova size (mg)	Estimated offspring size at birth (mg)	Reproductive allocation	Dry weight of reproductive tissue (gm)
<i>Poeciliopsis infans</i> (R. Verde)	0.99 (0.22)	26.78 (0.38)	1.4 (0.13)	6.14 (1.02)	0.86 (0.12)	0.85 (0.11)	0.09 (0.01)	0.01 (0.001)
<i>Poeciliopsis infans</i> (R. Ameica)	0.65 (0.12)	28.39 (0.56)	1.1 (0.10)	10.80 (1.58)	0.98 (0.11)	0.64 (0.07)	0.14 (0.02)	0.01 (0.003)
<i>Poeciliopsis infans</i> (R. Santiago)	0.66 (0.07)	30.41 (0.73)	1.1 (0.11)	10.09 (1.27)	0.96 (0.13)	0.77 (0.05)	0.10 (0.02)	0.01 (0.001)
<i>Poeciliopsis infans</i> (R. Ameica)	0.65 (0.06)	28.39 (0.52)	1.1 (0.14)	10.70 (1.24)	1.02 (0.08)	0.64 (0.04)	0.11 (0.01)	0.01 (0.001)
<i>Poeciliopsis lucida</i> (R. Sinaloa)	2.16 (0.27)	29.38 (0.87)	2.3 (0.15)	14.28 (1.72)	0.28 (0.03)	0.61 (0.04)	0.19 (0.02)	0.02 (0.003)
<i>Poeciliopsis lucida</i> (R. Fuerte)	1.31 (0.08)	31.92 (0.47)	2.1 (0.06)	9.27 (0.65)	0.77 (0.05)	1.01 (0.04)	0.10 (0.005)	0.02 (0.001)
<i>Poeciliopsis lucida</i> (R. San Pedro)	1.38 (0.07)	28.84 (1.05)	2.2 (0.14)	12.50 (1.14)	0.74 (0.03)	1.03 (0.06)	0.21 (0.02)	0.02 (0.003)
<i>Poeciliopsis occidentalis</i> (R. Matape)	1.16 (0.08)	35.45 (0.81)	2.0 (0.08)	4.40 (0.36)	1.78 (0.09)	2.08 (0.07)	0.08 (0.01)	0.02 (0.002)
<i>Poeciliopsis prolifica</i> (R. Presidio)	5.82 (0.13)	24.11 (0.96)	3.6 (0.14)	4.82 (0.60)	0.14 (0.01)	0.85 (0.16)	0.09 (0.01)	0.01 (0.001)
<i>Poeciliopsis prolifica</i> (La Huerta)	6.27 (1.69)	19.07 (0.39)	2.2 (0.49)	1.23 (0.15)	0.10 (0.01)	0.62 (0.12)	0.04 (0.01)	0.001 (0.0003)
<i>Poeciliopsis prolifica</i> (El Palillo)	6.30 (0.39)	24.63 (0.74)	3.2 (0.13)	4.42 (0.59)	0.12 (0.01)	0.78 (0.03)	0.08 (0.01)	0.004 (0.001)
<i>Poeciliopsis prolifica</i> (San Benito)	5.19 (0.43)	29.30 (1.38)	2.9 (0.17)	8.76 (0.91)	0.15 (0.01)	0.79 (0.06)	0.09 (0.01)	0.01 (0.002)
<i>Poeciliopsis monacha</i> (Juaguari)	0.84 (0.04)	25.72 (0.81)	1.5 (0.16)	4.48 (0.48)	2.37 (0.11)	1.99 (0.05)	0.28 (0.01)	0.03 (0.005)
<i>Poeciliopsis monacha</i> (Rio Fuerte)	0.81 (0.08)	34.46 (0.92)	1.8 (0.14)	7.78 (0.87)	2.16 (0.10)	1.73 (0.1)	0.13 (0.02)	0.03 (0.002)
<i>Poeciliopsis virosa</i> (Arroyo Las Palmas)	0.88 (0.05)	27.02 (0.57)	1.7 (0.08)	7.59 (0.56)	1.02 (0.04)	0.90 (0.03)	0.13 (0.01)	0.01 (0.001)
<i>Poeciliopsis virosa</i> (R. Presidio)	0.68 (0.05)	33.38 (1.49)	1.2 (0.17)	12.23 (1.9)	2.15 (0.08)	1.47 (0.08)	0.12 (0.01)	0.03 (0.001)

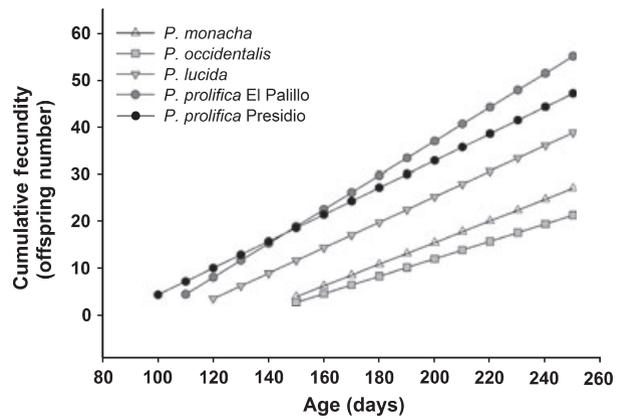


Fig. 3. Projected cumulative fecundity for *Poeciliopsis* based on mean age at maturity, mean litter size and mean interbrood interval values per taxon.

earlier age and reproduce more frequently; the time interval between successive litters is around half that of *P. monacha* (6.9 and 7.7 days for the two *P. prolifica* populations vs. 13.4 days for *P. monacha*). These shorter interlitter intervals are a consequence of *P. prolifica* carrying more simultaneously developing litters of embryos; i.e. it has a higher average degree of superfetation. As with all comparative studies, these observations represent correlations and hence do not constitute a demonstration of causality or adaptation (Leroi, Rose & Lauder 1994), but do form the basis for formulating a hypothesis for placental evolution consistent with well-established life-history theory (Rose 1983; Roff 1992, 2002; Stearns 1992).

Our results support one of the 'life-history facilitation' hypotheses, which is that the evolution of the placenta facilitates early maturation. Vitt & Blackburn (1983) and Blackburn, Vitt & Beuchat (1984) argued that placentation facilitated an early age at maturity in the highly placental species in this genus are known to ovulate at an early age and at a small body size that does not appear sufficient to support a litter of fully developed embryos (*Mabuya nigropunctata*, *Mabuya heathi* and *Mabuya frenata*: Vitt & Blackburn 1983, 1991; Vrcibradic & Rocha 1998). Embryos of *M. bistrata* and *M. heathi* were found to increase in dry mass by 47 400% and 38 400%, respectively, while the mother was able to grow substantially during the 9- to 12-month period of embryonic development. The prolonged duration of embryonic development gives females sufficient time to grow large enough to accommodate developing embryos. In contrast, lecithotrophic species must make their complete investment *before* an egg is fertilized and must be large enough at the time of fertilization to accommodate all developing young.

A more general significance of our results may lie in their relationship to existing life-history theory. Life-history theory predicts that earlier maturity and increased fecundity early in life will evolve in response to high extrinsic adult mortality rates (Gadgil & Bossert 1970; Law 1979; Michod 1979; Roff

1992; Hutchings 1993; Abrams & Rowe 1996). Such predictions have been supported in empirical studies on a diversity of organisms (e.g. Crowl & Covich 1990; Lüning 1992; Lafferty 1993; Sparkes 1996; Belk 1998; Clobert, Garland & Barbault 1998; Glazier 1999; Pijanowska *et al.* 2006). They are particularly well-documented in guppies (*Poecilia reticulata*) and other poeciliid fishes (e.g. Johnson 2001; Reznick & Ghalambor 2005; Jennions *et al.* 2006). The observed association between the degree of matrotrophy, the age at first parturition and the rate of reproduction early in life thus suggests a general, testable prediction: if the evolution of a placenta facilitates the evolution of earlier maturity, an increased level of superfetation, and hence an increase in the rate of reproduction early in life, then the evolution of placentotrophy will be favoured as part of a suite of life-history adaptations to high extrinsic adult mortality rates.

There is a key difference in how earlier age at first parturition and increased fecundity is achieved in *Poeciliopsis* in comparison with guppies or other poeciliids (*Brachyrhaphis episcopi* and *Brachyrhaphis rhabdophora*; Jennions *et al.* 2006; Johnson 2001) exposed to high-predation environments. These other species lack superfetation and matrotrophy, so the only way to increase fecundity early in life is to begin reproduction at an earlier age and to produce larger litters. Producing more offsprings at once will constrain how early they can mature, given that size and fecundity are highly correlated, and requires having higher reproductive allotments, i.e. a larger ovary. Evolving high fecundity in this fashion thus incurs the cost of reduced swimming performance (Ghalambor, Reznick & Walker 2004), which in turn may increase the probability of not being able to escape an attack by a predator (Walker *et al.* 2005). Northern Clade *Poeciliopsis* accomplish the same shift in reproduction, but with a *reduction* in reproductive allotment relative to close relatives that lack placentas and carry fewer simultaneously developing litters. For example, the RA of *P. prolifica* is less than half that of *P. monacha* (Table 1). They thus may increase offspring production early in life without paying the same cost in terms of reduced swimming performance as seen in guppies. We see similar trends in the field collections; ovary mass is most strongly correlated of all life-history traits with MI in both the bivariate and multivariate analyses.

Placentotrophy reduces average offspring size during the reproductive cycle because the egg size at fertilization is smaller than in lecithotrophic species (Table 1). Superfetation partitions what would otherwise be a single, large litter into multiple, smaller litters. By combining placentotrophy and superfetation (and, consequently, shorter interlitter intervals), the total number of offspring that a female is carrying is divided into smaller litters consisting of smaller embryos that are early in development and larger embryos that are later in development. The average total mass and volume of developing young is smaller than would be the case if all young were in the same stage of development. Therefore, the greater the degree of placentotrophy and superfetation, the less mass and volume will be required for an equal number of developing young. For an equal number of developing embryos, the loco-

motor cost of reproduction should also tend to be reduced as the degree of placentotrophy and superfetation increase. If the same association between RA and swimming performance seen in guppies is also found in *Poeciliopsis*, then this argument suggests that *Poeciliopsis* can adapt to high extrinsic mortality rates without incurring the same trade-offs with locomotor performance that have been documented in guppies.

The greatest limitation of our inferences is that we are evaluating a single instance of placental evolution. If the 'life history facilitation hypothesis' or the 'extrinsic mortality hypothesis' is general to placental evolution, then we should see the same pattern in studies including different clades representing independent origins of the placenta. The virtue of the genus *Poeciliopsis* is that it provides us with an opportunity to pursue such studies; the Southern Clade of this genus contains an independent origin of the placenta and includes close relatives that lack placentas (Reznick, Mateos & Springer 2002). Furthermore, placentas have evolved additional times elsewhere in the family Poeciliidae (e.g. Arias & Reznick 2000; Schrader & Travis 2005; Reznick, Meredith & Collette 2007), thus providing us with additional material for such comparative studies. This availability of multiple independent origins of a complex trait in a single family will enable us to develop a more robust scenario for the adaptive significance of placental evolution.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix S1. Collection information for the populations of wild-caught and lab-reared *Poeciliopsis* specimens used in this study.

Appendix S2. Feeding schedule, food rations and food effects for the laboratory study.

Appendix S3. Full correlation matrix of all life-history variables included in the study.

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