

# Pre- and post-fertilization maternal provisioning in livebearing fish species and their hybrids (Poeciliidae: *Poeciliopsis*)

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## Summary

1. Viviparous organisms vary greatly in their level of post-fertilization maternal provisioning and extensive provisioning (including very complex placentas) has evolved dozens of times in vertebrates. Through the use of species representing transitional states we aim to explore and develop empirically supported hypotheses that explain how such a complex trait has evolved.
2. In this study, we investigated the life histories and patterns of post-fertilization maternal provisioning in naturally occurring, all-female, hybrids between *Poeciliopsis monacha*, a viviparous species that produces large eggs and nourishes its embryos via stored yolk (lecithotrophy) and *P. lucida*, which produces small eggs and nourishes its embryos after fertilization via a follicular placenta (matrotrophy). Hybrids can be diploid or triploid, leading to different levels of gene dosage from the parental species.
3. We found that the extent of superfetation and average brood size of the hybrids did not differ significantly from their sexual parental species even though the latter was much lower in the hybrids. Moreover, the allodiploid, *P. monacha-lucida*, has intermediate sized eggs, whereas the allotriploid, *P. monacha-monacha-lucida*, produces eggs of similar size to those of *P. monacha*. The degree of post-fertilization maternal provisioning was unrelated to genomic dosages, with wild-caught hybrids being lecithotrophic, as seen in the maternal species (*P. monacha*).
4. Our results with naturally occurring *P. monacha-lucida* hybrids differed from those obtained with laboratory-synthesized hybrid clones, which were either lecithotrophic or weakly matrotrophic. Consequently, we propose two non-mutually exclusive hypotheses to explain why lecithotrophic clones might be favoured in the wild. One hypothesis suggests that lecithotrophic hybrids minimize competition with the matrotrophic paternal sexual species from which they require sperm to reproduce. Alternatively, we propose that natural selection has eliminated matrotrophic hybrids that suffer from intergenomic conflict in the degree of post-fertilization maternal provisioning.
5. We have thus identified an empirical system and developed testable hypotheses that will help elucidate the selective pressures that caused the evolution of post-fertilization maternal provisioning and potentially provide an opportunity to explore how epigenetic factors (e.g., intergenomic conflict) might influence this complex trait.

**Key-words:** asexual reproduction, frozen niche-variation model, genomic conflict, interspecific hybrids, placenta, placentotrophy, two-fold cost of sex, viviparity

## Introduction

Post-fertilization maternal provisioning varies greatly among viviparous organisms, ranging from strictly lecithotrophic species that produce mature eggs with a yolk supply sufficient for all embryonic and fetal development to matrotrophic species that produce eggs with minimal yolk and provide most

nutrients via a placenta (matrotrophy or ‘mother feeding’, Turner 1940; Blackburn 2000; Thompson *et al.* 2002) or other structures. Transitional states between these extremes have received less attention, however, and may provide a useful framework for investigating how and why placenta-like structures have evolved in a wide range of animals (Reznick, Mateos & Springer 2002). Extensive matrotrophy has evolved at least 24 times among the vertebrates and most often in fishes, yet no robust and empirically supported hypotheses

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currently exist for the evolution of post-fertilization maternal provisioning (Blackburn 1992; Trexler & DeAngelis 2003; Crespi & Semeniuk 2004). Nonetheless, knowledge of placental form and function derives mostly from studies of mammals, in which this structure is described as having the most variable morphology of any mammalian organ (Wourms, Grove & Lombardi 1988; Crespi & Semeniuk 2004). Its variable characteristics have been interpreted as byproducts of parent-offspring conflict engendered by the intimate physical contact between maternal and offspring tissues and differences in their genotypes (Haig 1993; Crespi & Semeniuk 2004; Vogel 2005). The optimal quantity of maternal resources demanded by individual offspring may exceed what is in the mother's best interest (Trivers 1974). Haig (1993) envisions an evolutionary 'tug-of-war' over the expression of genes that increase a fetus's capacity to gain a greater share of maternal resources versus genes that confer more control over the allocation of these resources by the mother. Any animal species with placentotrophic development could experience this conflict, especially when food is limited.

A fruitful approach for studying placentotrophic evolution is to concentrate on taxa that vary in the mode and extent of post-fertilization maternal provisioning, such as the livebearing fishes (Thibault & Schultz 1978; Reznick *et al.* 2002). The genus *Poeciliopsis* (Cyprinodontiformes: Poeciliidae) is particularly well suited for such studies because various species range from strictly lecithotrophic to extremely matrotrophic. Fertilization and development occur in the egg follicle. Species with extensive post-fertilization provisioning have elaborated the follicular membranes surrounding eggs into complex 'pseudoplacentas' that are responsible for the transfer of respiratory gases and nutrient (Turner 1940). In turn, the embryos of various species have developed specialized cells with microvilli, elaborated highly vascularized pericardial membranes, or expanded and vascularized yolk sacs (reviewed in Turner 1940; Wourms *et al.* 1988). Pseudoplacentas have evolved independently at least three times in *Poeciliopsis* and the extent of placentotrophy varies greatly among closely related species (Thibault & Schultz 1978; Schultz 1989; Reznick *et al.* 2002). At one extreme lies the strictly lecithotrophic species *P. monacha*, whose embryos lose 40% dry weight during development, and at the other extreme lies *P. retropinna*, a highly placentotrophic species whose embryos increase 11 700% in dry weight (Reznick *et al.* 2002).

Hybridization and clonal reproduction are unusual features that have made *Poeciliopsis* a useful model for genetic, ecological and evolutionary studies (Vrijenhoek 1994). The lecithotrophic species, *P. monacha* Miller (abbreviated *M*), has hybridized with a placentotrophic species, *P. lucida* Miller (abbrev. *L*) to produce naturally occurring, allodiploid (*ML*) and allotriploid (*MML* and *MLL*), all-female hybrids (or 'biotypes') that differ in their respective dosages of *M* and *L* genomes (Schultz 1989). Expression of maternal provisioning in the hybrids may provide insights into genetic control of this trait. For example, some traits like dentition, egg sizes, and cannibalistic behavior also differ between *P. monacha* and *P. lucida*, and they vary in an additive fashion in the hybrids according to dosages of

the *M* and *L* genomes (Schultz 1969; Thibault 1974a). The additive traits may well be under polygenetic control in these fish, but other traits appear to be oligogenic, such as genital pigmentation and vertebral number, because they exhibit various degrees of dominance (Schultz 1969). A useful feature of the allodiploid and allotriploid biotypes for ecological studies is the clonal diversity that they exhibit in nature (Vrijenhoek 1979), but perhaps their greatest utility resides in the relative ease in which new clonal diploid hybrids can be synthesized in the laboratory, clones that have not been 'purified' yet by natural selection (Wetherington *et al.* 1989).

Lima (2005) exploited the latter benefit in her study of post-fertilization maternal provisioning in laboratory-synthesized *ML* strains. She examined eight strains, and found two that were matrotrophic (embryos gained 27.0 and 28.8% dry weight during development) and six that were variably lecithotrophic (embryos lost 6.9 to 25.9% dry weight). The only source of genetic variance among these *ML* strains resides in the *M* genomes they 'froze' when they were produced by hybridization from genetically variable *P. monacha* mothers (Wetherington *et al.* 1989). The *L* genomes were identical because the male parent of all eight strains was an isogenic laboratory strain of *P. lucida*. Lima's study of these laboratory strains clearly revealed heritable variation for maternal provisioning among the *P. monacha* ancestors of the synthetic *ML* strains. Furthermore, the study showed that lecithotrophy is not a dominant trait in these hybrids. Nonetheless, she examined two naturally occurring *ML* strains that were lecithotrophic under laboratory conditions. She did not examine the expression of maternal provisioning in wild-caught *ML* strains, in the corresponding allotriploids, *MML* and *MLL*, or their sexually reproducing progenitors *P. monacha* and *P. lucida*. Consequently, it is not possible to determine whether long periods of laboratory culture of these animals might have contributed to artificial selection among clones that skewed her results. Nevertheless, inbreeding was not a problem in these laboratory strains, as the allodiploids and allotriploids are fixed heterozygotes in a large proportion of their genetic loci (Vrijenhoek, Angus & Schultz 1978; Quattro, Avise & Vrijenhoek 1992).

Here we report on the extent of variation for maternal provisioning in wild all-female populations of *Poeciliopsis* and their sexual progenitors. To address the potential effects of genomic dosage, we compared the all-female hybrids to sexual progenitors from the same locality. If the wild hybrids express a range of variation similar to that of laboratory-synthesized hybrids, we might infer that differences in maternal provisioning are products of genetic variation that are 'frozen' from variable sexual ancestors when the clones first arose, as proposed in the 'frozen niche-variation' model (Vrijenhoek 1979, 1984). Nonetheless, wild-caught hybrid clones are survivors of many generations of natural selection. If the wild hybrids express limited variation in maternal provisioning relative to the laboratory hybrids, we might infer that purifying selection has favoured a particular range of expression for this trait. The present comparisons of wild-caught fish provide a first step for creating testable hypotheses about placental evolution in *Poeciliopsis*.

## Materials and methods

### STUDY SYSTEM

Natural hybridization between *P. monacha* and *P. lucida* has produced all-female, clonally reproducing fish that are highly successful in the desert streams of northwestern Mexico (Schultz 1989). Multiple hybridization events have given rise to diverse clonal lineages that can be identified with various genetic methodologies including tissue grafting, multilocus allozymes, and mitochondrial DNA (Vrijenhoek *et al.* 1978; Quattro, Avise & Vrijenhoek 1991; Quattro *et al.* 1992). The allotriploid biotypes *P. monacha-lucida-lucida* (MLL) and *P. monacha-monacha-lucida* (MML) reproduce by gynogenesis. Sperm from a coexisting sexual species is required to activate embryogenesis, but the all-female progeny are exact clones of the mother (Cimino 1972b). The allodiploid biotype, *P. monacha-lucida* (ML), reproduces by hybridogenesis (Schultz 1969). ML females produce haploid eggs that contain only the clonally inherited *M* genome (Cimino 1972a). True insemination by males of *P. lucida* is required to restore the ML genotype in each generation, so inheritance in these fish is 'hemiclinal' (Vrijenhoek *et al.* 1978).

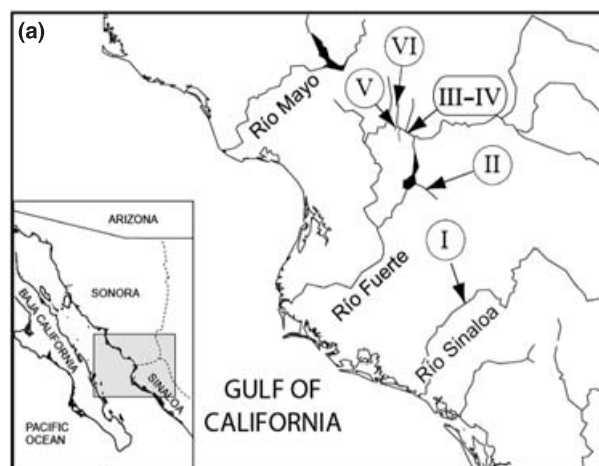
### SAMPLING AND BIOTYPE IDENTIFICATION

Fish were collected from Pacific drainages in Northwestern Mexico (Fig. 1a). Specimens from Río Sinaloa (site I: 25°50' N; 108°15' W) were collected in 1955 by R.R. Miller and J.T. Greenbank (UMMZ 172270). Specimens from the San Pedro tributary of the Río Fuerte (site II: 26°30' N; 108°30' W) were collected in 1961 by R.R. Miller and H.L. Huddle (UMMZ 179777). Specimens from all other Río Fuerte sites were collected during March and April of 1981 by R. Vrijenhoek: sites III and IV (26°53' N; 108°38' W) from neighboring pools in the Arroyo de Jaguari mainstream; site V (26°54' N; 108°40' W) from Arroyo de Nachapulon; and site VI (26°57' N; 108°40' W) from Aguajita de Tarahumara (Fig. 1a). R.R. Miller and H. L. Huddle using detailed morphological measurements confirmed the identity of the two earlier collections of *P. lucida*. For the 1981 collections, allozymes polymorphisms at three loci (*Ldh-1*, *Idh-2*, and *Pgd*) unambiguously discriminated among *P. lucida*, *P. monacha*, and clonal variants of ML (hemiclones VIII and VIII) and MML (clones I and II). Refer to Schenck & Vrijenhoek (1986) for allozyme methods and a detailed seasonal analysis of clonal variation in this tributary of the Río Fuerte.

The samples available for this study did not contain the MLL biotype, so our comparisons only involved the two parental species and the ML and MML hybrids. Not all fish types (sexual species, all-female biotype or clone) were represented in all collections, so there is some potential for confounding fish type differences with environmental differences among collections (Fig. 1b), yet, this should not be a serious limitation for two reasons. First, the two main *P. lucida* collections were made six years apart and in different rivers, yet show no collection heterogeneity for maternal provisioning. Second, *P. lucida* from the 1981 collections fell within the range of values of the earlier collections for all traits.

### LIFE-HISTORY CHARACTERIZATION

Life-history attributes of females were obtained by dissection without knowing the fish type for the 1981 collections. Fish types were matched afterwards. We used a classification system modified from Haynes (1995) for embryonic development that classified the embryos into six stages of development plus five additional categories that fell



(b)

Site	Year	<i>P. monacha</i>	MML	ML	<i>P. lucida</i>
I	1955				20
II	1961				19
III	1981	1		3	2
IV	1981	7	2	27	4
V	1981	3	13		
VI	1981	24	5		
Total		35	20	30	45

Fig. 1. (a) Collection localities in Northwestern Mexico and (b) the number of females with developing broods of each species and biotype collected at each site.

between these stages. These eleven categories were then assigned numerical values that ranged from zero (unfertilized fully-yolked egg) to 50 (fully-developed embryo at birth) in 11 five-unit steps. This scale was first used by Reznick (1981) to describe the patterns of mass change through development in *Gambusia affinis* and *G. holbrooki*, but it yields similar results for *Poecilia reticulata* (Reznick & Endler 1982) and some species of *Poeciliopsis* (Reznick *et al.* 2002). We retain this scale because it provides a frame of reference for comparisons among poeciliid taxa. Each developing brood was assigned to one of these 11 stages of development. Its dry weight was recorded after 24 hours in a drying oven. Mean embryonic dry weight was calculated as the brood's total dry weight divided by the number of embryos.

We quantified maternal provisioning by determining the change in dry weight of broods through development. Lecithotrophic species typically register a 30–40% loss of dry mass between fertilization and birth, whereas placental species remain fairly constant or increase in dry mass during development (Reznick & Miles 1989). All species of *Poeciliopsis* have superfetation, an ability to gestate multiple broods at different stages of development (Turner 1940). The degree of superfetation was recorded as the number of simultaneously developing broods. Brood size for each female was calculated as the average brood size of all developing broods. Yolking ova or broods without visible embryonic development (stage 0) were excluded from the analyses due to difficulties in correctly discriminating between fully yolked and immature eggs.

### STATISTICAL ANALYSES

We used a generalized linear mixed model (GLMM of SPSS v.15) to assess differences among the fish types (species, biotypes and clones)

for maternal provisioning and egg size at fertilization. The dependent variable was  $\ln$ -transformed mean embryonic dry weight of each brood, the fixed effects were fish type, brood stage of development (covariate), and their interaction, and the random effects were sample collection and the interaction between collection and the covariate. Significant differences in intercepts indicate that egg sizes differ among the fish types. Significant differences in the interactions between fish type and developmental stage indicate differences in degrees of maternal provisioning. To estimate offspring size at birth, we extrapolated the GLMM parameter estimates of each species to developmental stage 45 (late-development embryos, a conservative estimate). The matrotrophy index (the estimated dry mass of offspring at birth divided by the estimated dry mass of eggs at fertilization) is reported to facilitate comparisons with studies of other poeciliids (Reznick *et al.* 2002).

Untransformed values for the degree of superfetation and average brood size were analyzed separately with the same GLMM models. The fixed effects were fish type, body length (covariate), and their interaction and collection was included as a random effect. A quasipoisson distribution best fit the error residuals and thus we used the glmmPQL procedure in R (GUI 1.22 for Macintosh computers) with a quasipoisson error distribution. This procedure  $\ln$ -transforms the independent variable.

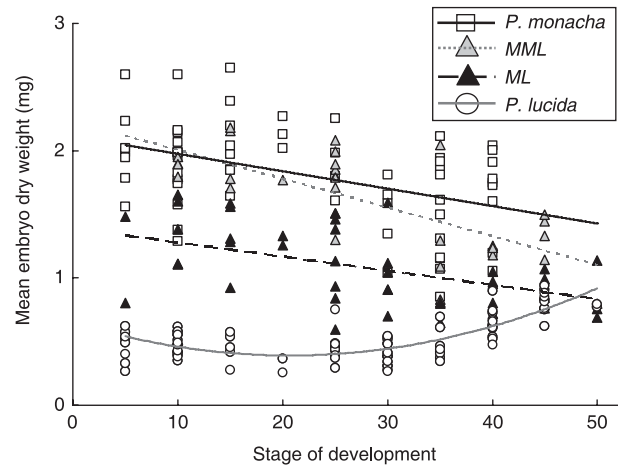
## Results

### CLONAL ANALYSES

Allozyme analysis of the 1981 sample identified two clonal lineages of the allotriploid biotype (*MML/I* and *MML/II*) and two hemiclinal lineages of the allodiploid biotype (*ML/VII* and *ML/VIII*). Based on GLMM procedures, the clonal lineages within each biotype did not differ significantly with respect to egg sizes ( $t$ -test for each clonal lineage,  $t_{ML} = -0.01$ ,  $P_{ML} = 0.993$  and  $t_{MML} = -0.80$ ,  $P_{MML} = 0.429$ ) or maternal provisioning (slope)  $t_{ML} = 0.23$ ,  $P_{ML} = 0.818$  and  $t_{MML} = 0.87$ ,  $P_{MML} = 0.394$ ). The clonal lineages also did not differ with respect to superfetation ( $t_{ML} = 0.56$ ,  $P_{ML} = 0.58$  and  $t_{MML} = 1.78$ ,  $P_{MML} = 0.097$ ) or average brood size ( $t_{ML} = -0.97$ ,  $P_{ML} = 0.34$  and  $t_{ML} = 0.87$ ,  $P_{MML} = 0.40$ ). The absence of statistically significant differences for these traits between clones justified lumping them within a biotype for subsequent analyses.

**Table 1.** Parameter estimates of generalized linear mixed model analysis of  $\ln$ -transformed mean embryo weights. Intercepts represent egg size whereas the covariates and their interactions represent patterns of maternal provisioning. Intercept is biotype *ML*. (StgDev. = Brood stage of development)

Fixed effects	Estimate	Standard Error	df	<i>t</i>	<i>P</i> -value
Intercept	0.269	0.115	18.7	2.34	0.030
<i>MML</i>	0.381	0.149	194.9	2.57	0.011
<i>P. lucida</i>	-1.174	0.131	61.6	-8.93	0.000
<i>P. monacha</i>	0.355	0.115	176.3	3.10	0.002
StgDev.	-0.0096	0.0036	13.4	-2.64	0.020
<i>MML</i> * StgDev.	-0.0025	0.0050	108.5	-0.49	0.626
<i>P. lucida</i> * StgDev.	0.0225	0.0041	31.5	5.44	0.000
<i>P. monacha</i> * StgDev.	-0.0037	0.0041	89.3	-0.89	0.375
Random effects	Variance	Standard error			
Residual	0.0503	0.0049			
Collection	0.0258	0.0230			
Collection × StgDev.	1.7000 E-5	2.150 E-5			



**Fig. 2.** Change in embryonic dry weight during embryonic development for the parental sexual species and unisexual hybrids.

### MATERNAL PROVISIONING

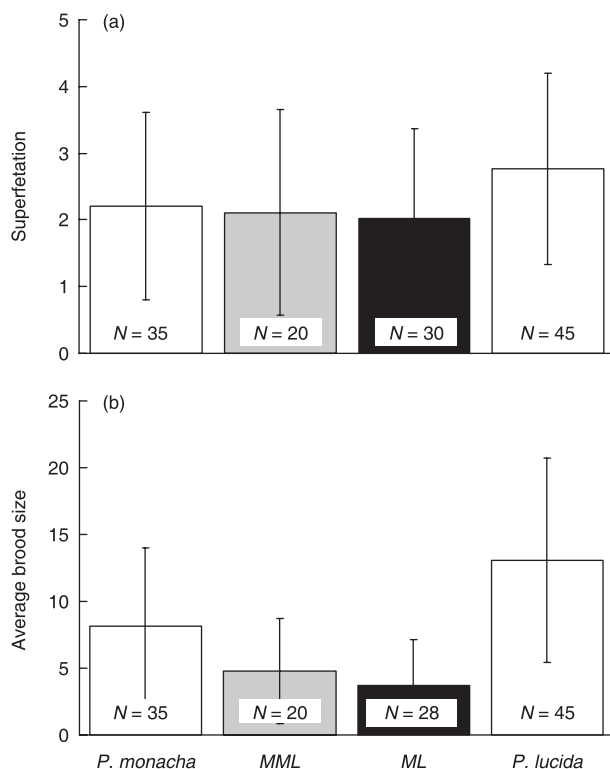
The pattern of maternal provisioning in the parental species followed the pattern described in previous studies (Reznick *et al.* 2002). *P. lucida*'s embryos gain dry weight through development in a manner best described by a quadratic function whereas *P. monacha*'s embryos lose dry weight during development in a linear manner (Fig. 2). The patterns of maternal provisioning in *ML* and *MML* hybrids were statistically identical to that of *P. monacha* (equivalent slopes), which differed significantly from that of *P. lucida* (Table 1; Fig. 2).

### EGG AND EMBRYO SIZE

*P. lucida*'s eggs at fertilization are approximately one third to one quarter of the other fish types' and gain 79% weight through development yet still produce the smallest offspring (Table 2). *P. monacha* have large eggs at fertilization that lose 45% weight over embryonic development. The *ML* hybrid, with 50% *P. monacha* genomic composition, is intermediate

**Table 2.** Life-history characteristics of both parental sexual species and unisexual hybrids extracted from the analysis in Table 1. Offspring size at birth corresponds to estimated mass for late embryonic development (stage 45). Superscripts indicate significant differences between fish types

Fish types	Percent <i>P. lucida</i>	# of broods weighed	Egg size at fertilization (mg)	Offspring size at birth (mg)	Matrotrophy index
<i>P. lucida</i>	100%	90	0.404 <sup>a</sup>	0.723 <sup>a</sup>	1.79
<i>ML</i>	50%	44	1.309 <sup>b</sup>	0.849 <sup>b</sup>	0.65
<i>MML</i>	33%	29	1.916 <sup>c</sup>	1.113 <sup>c</sup>	0.58
<i>P. monacha</i>	0%	62	1.866 <sup>c</sup>	1.027 <sup>c</sup>	0.55

**Fig. 3.** Untransformed predicted means from the generalized linear mixed model analysis for (a) the degree of superfetation and (b) average brood size for each parental sexual species and unisexual hybrids at a common mean body length of 32.43 mm. Sample sizes are indicated within the bars. Error bars represent one standard error.

in both egg size and offspring size between the parental species. The eggs and offspring of the *MML* hybrid, with 66% *P. monacha* in genomic composition, are statistically equivalent to those of *P. monacha* (Table 2).

#### SUPERFETATION AND BROOD SIZE

Fish types did not vary significantly in the degree of superfetation (intercepts) or in the relationship between the degree of superfetation and body length (GLMM,  $df = 117$ , all  $P > 0.35$ , Fig. 3a). Although average brood size seemed to differ between fish types (Fig. 3b), differences in intercept and the relationship between mean brood size and body length are not significant (GLMM,  $df = 115$ , all  $P > 0.15$ ).

#### Discussion

The genus *Poeciliopsis* is well-known for its impressive variation in reproductive modes (Thibault & Schultz 1978; Reznick *et al.* 2002). The lecithotrophic species, *P. monacha* (*M*), has relatively large eggs that lose about 45% dry weight during development; whereas the placentotrophic species, *P. lucida* (*L*), has small eggs that nearly double in dry weight during embryonic development. Hybridization between the two species has produced allodiploid (*ML*) and allotriploid (*MML*) all-female biotypes that exhibit a range of expression for traits involved in maternal provisioning. The sizes of mature eggs and offspring at birth were roughly intermediate in the *ML* hybrids, suggesting additive polygenic effects due to the *M* and *L* genomes. However, the *MML* hybrids were not intermediate and did not differ significantly from *P. monacha* for egg or offspring sizes. Perhaps the possession of two copies of the *M* genome exceeds a dosage threshold that suppresses expression of *L* genome for these traits. The *MML* biotype was not present in our samples, so we could not ascertain its expression of these traits. The pattern of maternal provisioning as quantified by the change in embryonic weight and the matrotrophy index exhibited complete dominance in the *ML* and *MML* hybrids, as both of these biotypes were identical with *P. monacha* (Table 1 and 2). None of the four fish types differed significantly in superfetation (extent of overlapping broods; Fig. 3a). The present results add to a growing body of evidence that the degree of matrotrophy varies independently of the extent of superfetation among poeciliids (Thibault & Schultz 1978; Reznick & Miles 1989). The hybrids examined in this study appeared to produce broods that were about half the size of the sexual broods (Fig. 3b), but the differences were not statistically significant. Earlier field studies revealed that the all-female biotypes lag seasonally behind the sexuals in their reproductive peaks (Thibault 1974b).

Though genomic dosages are partially related to pre-fertilization egg size in these hybrids, the *P. monacha* (lecithotrophic) genome behaves in a purely dominant fashion with respect to post-fertilization maternal provisioning. Three hypotheses might explain the latter result: (1) lecithotrophy is strictly dominant to matrotrophy; (2) maternal provisioning is maternally inherited; and (3) maternal provisioning is shaped by natural selection via the differential survival of hybrid clones. Lima's (2005) observation that *ML* hybrids synthesized *de novo* in the laboratory could be either lecithotrophic or



matrotrophic allows us to reject the first two hypotheses. The allotriploids, *MML* and *MLL*, have not been generated in the laboratory; consequently, the expression of maternal provisioning in *de novo* triploids is unknown. Additionally, we were unable to examine these traits in wild-*MLL* hybrids, because they were not found in our samples from the Río Fuerte. It would be of interest to determine whether a single genome from a lecithotrophic species could countermand two genomes from a matrotrophic species.

Lima's (2005) experiments revealed that clonally reproducing, matrotrophic hybrids of *P. monacha* and *P. lucida* can be generated *de novo* in the laboratory, but we found no evidence for matrotrophic hybrids in nature. The hybrid lineages that we studied, two hemiclonal strains of the allodiploid, *ML*, and two clones of the allotriploid, *MML*, had statistically identical patterns of maternal provisioning and did not differ from *P. monacha*. It is possible, therefore, that post-formatonal natural selection has determined the level of maternal provisioning that can persist among the naturally occurring hybrids. Other evidence for post-formatonal natural selection is seen in sexual mimicry by the hybrids (Lima, Kobak & Vrijenhoek 1996). To reproduce, hybridogenetic *ML* females need sperm from 'choosy' *P. lucida* males (Moore & McKay 1971). Genital pigmentation patterns of wild-*ML* hybrids closely resemble those of *P. lucida* females, whereas laboratory-synthesized *ML* strains range widely in their expression of these pigments. Theoretically, the poor mimics should disappear quickly in nature as they would be less successful in acquiring insemination by 'choosy' *P. lucida* males (Lima *et al.* 1996).

Other selective pressures may favour divergence of *ML* strains from their sexual hosts. For example, lecithotrophic *ML* hybrids in nature may be products of selection for clones that have limited resource overlap with their matrotrophic *P. lucida* sexual hosts. Coexistence between the sperm-dependant sexual parasites and their hosts is facilitated by ecological diversification that minimizes resource competition (Vrijenhoek 1979; Kirkendall & Stenseth 1990; Schley, Doncaster & Sluckin 2004). According to the *frozen niche-variation* model, natural selection will favour invasive (or *de novo*) clones that have minimal niche-overlap with their sexual hosts (Vrijenhoek 1984; Weeks 1993). Lecithotrophic forms are predicted to produce more offspring than matrotrophic lineages living in environments with highly variable food resources (Thibault & Schultz 1978; Trexler & DeAngelis 2003). Small invertebrates (insect larvae, ostracods, copepods, etc.) are most concentrated in the diets of these fish during the late spring as the annual dry season progresses and residual pools shrink in volume (Schenck & Vrijenhoek 1986; Weeks *et al.* 1992). These invertebrates become overdispersed and less accessible to the fish during the rainy season when streams are rapidly flowing. The matrotrophic species, *P. lucida*, is primarily a deposit feeder, eating detritus, a low-energy resource that is nevertheless always available. In contrast, the lecithotrophic species, *P. monacha*, is an opportunistic predator that shifts its diet to high-energy invertebrates when they are seasonally abundant (Schenck & Vrijenhoek 1986; Weeks

*et al.* 1992). All the hybrid biotypes studied to date are more predaceous than *P. lucida*. It is conceivable, that selection favours *ML* hybrids that feed more on ephemeral food resources, such as the seasonally abundant small invertebrates, to reduce competition with *P. lucida*. Such selection would also favour hybrid clonal lineages that are lecithotrophic if this trait is functionally or genetically linked to foraging strategy. Whether this is true or not remains to be empirically determined.

Alternatively, we also consider the possibility that the fixation of lecithotrophy in the natural hybrids may be driven by pressures to avoid parent-offspring conflicts. Embryos of placental vertebrates, including fish (Hamlett 1999), can influence maternal reproductive investment through steroid and prostaglandin secretions (Guillette 1989; Haig 1993; Crespi & Semeniuk 2004). Selection should favour counter-adaptations that enable mothers to retain control over resource allocation (Haig 1993). Such conflicts are mitigated in lecithotrophic clones because maternal provisioning (i.e. yolk deposition) occurs prior to fertilization and hence prior to the expression of the paternal genome (Haig 1993; Crespi & Semeniuk 2004). Conversely, matrotrophic clones provision embryos that may express conflicting interests if their genotypes differ from that of the mother. Such conflicts should not exist for the triploid gynogens, as inheritance is strictly clonal. Genotypes are identical and tissue grafts are readily accepted between mother and offspring of *MML* (Moore 1977). Nonetheless, maternal-fetal conflicts could exist in the hemiclonal *ML* lineages, because the paternal *L* genomes of these hybrids are substituted each generation and different from that of the mother (Vrijenhoek *et al.* 1978; Angus & Schultz 1979). Embryonic expression of the *L* genome, which is provided by the matrotrophic species *P. lucida*, could generate conflicts between the mother's ability to control resource allocation and her offspring's ability to acquire them. Since the *L* genome is not heritable in the hybrids, selection cannot act to alter its expression, but interclonal selection can act to favour the heritable *M* genomes that suppress matrotrophy and its potentially detrimental effects to the mother.

Whether the fixation of lecithotrophy in natural *ML* hybrids is a product of pressures to avoid maternal-offspring conflict and/or to avoid niche overlap remains to be empirically resolved. Nonetheless, the variation seen among these and other *Poeciliopsis* species and the opportunity to generate *de novo* clones in the laboratory provide rare opportunities to experimentally study potential selective pressures associated with the evolution of placentation. Because the *MML* and *MLL* allotriploids are 'true' clones and will not conflict genetically with their mothers, whereas the *ML* allodiploids are hemiclones and can conflict with their mothers, these fish may provide a unique opportunity to examine epigenetic control over genes thought to be involved in avoidance of such conflicts. Studies into these possibilities have just begun (O'Neill *et al.* 2007).

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