Chapter 3 Variation and evolution of reproductive strategies

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3.1 Introduction

THE FAMILY POECILIIDAE (Rosen & Bailey 1963) consists of a well-defined, monophyletic group of nearly 220 species with a fascinating heterogeneity in life-history traits. Reznick and Miles (1989a) made one of the first systematic attempts to gather information from a widely scattered literature on poeciliid life histories. They focused on two important female reproductive traits: (1) the ability to carry multiple broods at different developmental stages (superfetation; Turner 1937, 1940b, 1940c), which tends to cause females to produce fewer offspring per brood and to produce broods more frequently, and (2) the provisioning of eggs and developing embryos by the mother, which may occur prior to (lecithotrophy) or after (matrotrophy) fertilization. Their review documented the distribution of these two reproductive traits among poeciliids and their correlates with other life-history traits, including mean and minimum reproductive size, reproductive allocation, brood size, offspring dry weight at birth, interbrood interval, and mean adult size.

Their study yielded several new insights. One was that superfetation may have evolved multiple times within the family and appeared to be largely confined to the closely allied genera *Heterandria*, *Neoheterandria*, and *Poeciliopsis*. The authors cautioned, however, that their data set was limited by uneven representation of genera. Further knowledge of other, hitherto-uninvestigated genera was required before reaching a conclusion that superfetation is confined to these three genera. A second insight was that most species with superfetation were matrotrophic; the strong as-sociation between these two traits suggests that one of the two traits might be more likely to evolve when the other trait is already present (the latter facilitating the evolution of the former). However, the existence of a notable exception in the literature (the lecithotrophic, superfetating *Poeciliopsis monacha*, the only known exception at the time) showed that superfetation and matrotrophy were not strictly linked, indicating that these two traits can evolve independently of each other.

Reznick and Miles (1989a) also proposed a framework for future research that was aimed at evaluating possible causes and mechanisms for the evolution of superfetation and matrotrophy by (1) gathering detailed life-history descriptions of a greater number of poeciliid species, either through common garden studies or from field-collected individuals, (2) comparing superfetating species with their closest nonsuperfetating relatives (and matrotrophic species with their closest lecithotrophic relatives) to test hypotheses about the ecological conditions under which superfetation (or matrotrophy) should be favored, (3) developing a highly resolved, family-wide phylogenetic tree, and (4) applying phylogenetic comparative methods (e.g., phylogenetically independent contrasts; Felsenstein 1985) to test for correlations among different facets of reproductive modes (e.g., superfetation, matrotrophy), on the one hand, and to interpret either the descriptive life-history traits or the outcome of paired experiments, on the other.

Over the last 20 years, much action has been taken on these recommendations. Some of the published work will be synthesized in this chapter, and occasionally we will draw upon our own unpublished data. We concentrate on

the evolution of superfetation and postfertilization maternal provisioning (matrotrophy) and address these specific questions:

1. What are the ranges of variation in some traits that are associated with superfetation and postfertilization maternal provisioning (i.e., standard length, offspring size, brood size, and reproductive allocation) within the family Poeciliidae (section 3.2)?
2. To what extent do poeciliids vary in their ability to carry multiple broods (degree of superfetation) and in the amount of postfertilization maternal provisioning (degree of matrotrophy), and are these traits coupled (section 3.3)?
3. What are the patterns of life-history variation within the family, and what do they reveal about evolutionary trends of trait evolution in poeciliids (section 3.4)?
4. What are some of the available hypotheses for the evolution of postfertilization maternal provisioning and superfetation, and to what extent are they supported (section 3.5)?

3.2 Life-history variation in the family Poeciliidae

Poeciliids display wide variation in all life-history traits. Here, we briefly outline the ranges of variation of several life-history traits within the family Poeciliidae known (or suspected) to be associated with postfertilization maternal provisioning. The genera *Cnesterodon*, *Pamphorichthys*, *Phalloptychus*, and *Poecilia* (subgenus *Acanthophacelus*) contain some of the smallest known species in the family (e.g., mean standard length [SL] for *Cnesterodon iguape*, male = 19.7 mm, female = 21.4 mm; *Pamphorichthys pertapeh*, male = 15.9 mm, female = 18.04 mm; *Phalloptychus januarius*, male = 17.3 mm, female = 23.7 mm; *Poecilia picta*, male = 18.3 mm, female = 19.8 mm; Reznick et al. 1992; Rosa & Costa 1993; Lucinda 2005b, 2005a; Figueiredo 2008; Pires et al. 2010). On the other hand, the genera *Poeciliopsis, Poecilia*, and *Belonesox* have some of the largest poeciliid species (e.g., mean male/female SL of 72.4/102.9, 63.8/85.7, 36.0/83.5, and 35.0/66.0 mm for *Belonesox belizanus*, *Poecilia catemaconis, Poeciliopsis elongata*, and *Poeciliopsis catemaco*, respectively; Miller 1975; Turner & Snelson 1984; Reznick, unpublished data). Poeciliids typically show strong size dimorphism, with males being considerably smaller than females (Bisazza 1993a). Despite this size dimorphism, males display a variation in mean SL similar to that of females (approximately fivefold). Mature males within a population often display polymodal or highly skewed size distributions (Kallman 1989; Kolluru & Reznick 1996; Arias & Reznick 2000). In some of these

species, it has been shown that the different size modes correspond to genes linked to the Y chromosome that control the age and size at maturity (Kallman 1989).

Offspring size (like many other life-history traits) is typically related to female body size (Reznick & Miles 1989a); some of the smallest offspring are produced by the smallest species within the family. Most studies express offspring size in terms of embryo dry weight, which exhibits a 14-fold range of variation (mean neonate mass ranging from 0.58 mg in *Poeciliopsis prolifica* to 8.3 mg in *P. elongata*; Pires et al. 2007; Reznick, unpublished data). An increase in female body size is generally also associated with longer interbrood intervals in both superfetating and nonsuperfetating species (Reznick & Miles 1989a). In nonsuperfetating species, for instance, interbrood interval ranges from 21.9 days in the small-bodied *Poecilia reticulata* (Thibault & Schultz 1978) to 63.8 days in the larger *Poecilia latipinna* (Hubbs & Dries 2002). In superfetating species, there appears to be a similar trend: larger species, such as *Poeciliopsis gracilis* and *Poeciliopsis turneri*, which on average carry two to three broods simultaneously, have an interbrood interval of 11–23 days (Thibault & Schultz 1978; Snelson et al. 1986), while the somewhat smaller-bodied *Heterandria formosa* (Scrimshaw 1944b) and *Phalloptychus januarius* (Pollux, unpublished data) can give birth every 1–3 days to as few as one to five offspring at a time. Brood size (the number of offspring per brood) is related to female body size in nonsuperfetating species, with larger species (as well as larger individuals within species) on average producing larger broods than their smaller counterparts (e.g., Turner & Snelson 1984; Reznick & Miles 1989a; Reznick et al. 1992; Reznick et al. 1993). In superfetating species, however, brood size appears to be independent of female size, both within (e.g., *Xenodexia ctenolepis*; Reznick et al. 2007a) and among (Reznick & Miles 1989a) species. In these species, brood size seems to depend more on the degree of superfetation, with highly superfetating species expected to have smaller broods than similar-sized species that carry fewer simultaneous broods at a time (Reznick & Miles 1989a). Taking all poeciliids into account, there is a remarkable, nearly 100- to 200-fold, variation in mean brood size, ranging from 1 offspring per brood (e.g., *H. formosa*, *Phalloptychus januarius*, *P. prolifica, Gambusia hubbsi*; Scrimshaw 1944a; Downhower et al. 2000; Pires et al. 2007; Pollux, unpublished data) to over 99 young per brood in *B. belizanus* (Turner & Snelson 1984) and over 200 per brood in *Gambusia affinis* (Krumholz 1948).

Reproductive allocation (RA), defined as the percent of female dry weight that consists of developing embryos, shows a nearly ninefold variation in poeciliids, being as low as 4.1% in the matrotrophic *Phalloceros caudimaculatus*

(Arias & Reznick 2000) and over 35% in the lecithotrophic *G. affinis* (Stearns 1983c; Reznick & Braun 1987).

Finally, it is noteworthy that life-history traits can vary substantially within species, as a result of either maternally mediated environmental influences (phenotypic plasticity) or genetic differences among spatially isolated populations (local adaptation) (Stearns 1983b, 1983a; Trexler 1989; Johnson & Bagley, **chapter 4**).

3.3 Variations on the theme of livebearing

3.3.1 Maternal provisioning

All poeciliids exhibit internal fertilization, and all but one species, *Tomeurus gracilis*, are viviparous. Viviparity, or "livebearing," is a reproductive mode in which eggs are fertilized internally and then retained in the maternal reproductive system throughout embryonic development until parturition, resulting in a free-living offspring at birth (Wourms et al. 1988).

The lengthy maternal-embryonic interaction resulting from viviparity creates the need for respiratory, osmoregulatory, and endocrinological interactions between the mother and embryos. These intimate interactions in turn create the potential for the evolution of more complex trophic relationships (Wourms et al. 1988; Korsgaard & Weber 1989; Crespi & Semeniuk 2004). Maternal-offspring trophic relationships within poeciliids range from strict lecithotrophy, in which nutrients are provisioned to the embryo solely via yolk allocated to the egg prior to fertilization, to extensive matrotrophy, in which the developing embryo depends largely or completely on a continuous supply of nutrients obtained directly from the mother during gestation (Wourms et al. 1988).

Variation in maternal-offspring trophic relationships in poeciliids can be interpreted within the context of the capital versus income breeding paradigm for reproductive strategy variation, which distinguishes between species that use stored energy to nourish developing offspring (analogous to a lecithotrophic strategy) and species that provision offspring with current energy income (analogous to a matrotrophic strategy; Drent & Daan 1980). Rather than being a true dichotomy (Houston et al. 2007), these trophic modes represent extremes of a continuum in which the embryo relies on prefertilization (indirect) and postfertilization (direct) maternal provisioning in different degrees. Thus, in many poeciliids, lecithotrophy occurs in conjunction with matrotrophy: developing embryos obtain nutrients both from the yolk (prefertilization maternal provisioning) and directly from the mother (postfertilization maternal provisioning). For simplicity, however, species are referred to as "matrotrophic" when postfertilization maternal provision-

ing exists and is large enough to be reflected in some embryonic weight gain during development, while the term "lecithotrophic" is applied to species in which all or most nutrients are provided to the developing embryo before the egg is fertilized (as is the case in an oviparous species). The term "true viviparity" has been used to refer to matrotrophic viviparity (e.g., Scrimshaw 1944a; Trexler 1985), but it should be avoided because it confounds trophic relationships and parity mode (Blackburn 1992).

Matrotrophy is widespread in metazoans, and the mechanism of resource transfer varies greatly across different taxa (e.g., Wourms et al. 1988; Blackburn 1992; Meier et al. 1999; Williford et al. 2004). In poeciliid fishes, matrotrophy is accomplished through a specialized follicular placenta (the "follicular pseudoplacenta" described by Turner 1940b)—the close apposition of the follicle wall (the maternal tissue) to vascularized embryonic tissues (Wourms et al. 1988). The yolk sac is the main embryonic component of the placenta of lecithotrophic poeciliids and facilitates gas exchange and/or transfer of inorganic and a few organic molecules between the mother and the embryos (Wourms et al. 1988; Constantz 1989). In matrotrophic species, however, the yolk sac is greatly reduced, and a modified, highly vascularized pericardial sac allows substantial nutrient transfer between the mother and the embryo (Wourms et al. 1988). The term "placentotrophy" (Wourms et al. 1988; Blackburn 1992) will be used throughout the chapter to refer to this specific form of poeciliid matrotrophy. "Matrotrophy" will henceforth be used to refer to a more general form of postfertilization maternal provisioning.

Because many species have both pre- and postfertilization maternal provisioning, there can be intra- and interspecific variation in the degree of prefertilization yolk allocation (i.e., egg size and yolk composition) and of postfertilization placentotrophic allocation. Strictly lecithotrophic poeciliid species are characterized by a decrease of ca. 35% (ranging from 25% to 55%) in embryonic dry weight over development, which is similar to the change in embryonic dry weight during development observed in oviparous fishes (Wourms et al. 1988). Embryos from placentotrophic poeciliids, on the other hand, may obtain just enough postfertilization resources from the mother to compensate for biomass loss during development due to metabolic costs—in which case there will be no net change (or even a slight loss) in dry weight between the egg at fertilization and the embryo at birth. In the case of extensive placentotrophy, the gain in embryonic dry weight over development can exceed 10,000% (Reznick et al. 2002b).

Placentotrophy has been identified and quantified in poeciliids with the matrotrophy index (MI; Reznick et al. 2002b). The MI is the estimated dry weight of the offspring

at birth divided by the estimated dry weight of the egg at fertilization and thus represents the change in embryonic dry weight over development (Wourms et al. 1988; Reznick et al. 2002b; Stewart & Thompson 2003; Thompson & Speake 2006). An MI that is significantly greater than 0.7 or 0.8 (the upper threshold in oviparous species; Wourms et al. 1988) indicates that a placenta is transferring resources to embryos during development and thus characterizes functional placentotrophy. Placentotrophy may be present even if there is a slight loss in dry mass (i.e., MI slightly less than 1 but still significantly greater than 0.7 or 0.8). An MI of 1 thus represents no net change in dry weight over embryonic development.

Trophic patterns of all species in the genus *Poeciliopsis* have been characterized and reflect the diversity of trophic modes that can be found among closely related poeciliid species. There have been three independent origins of extensive placentotrophy in this genus alone (Reznick et al. 2002b). Lecithotrophic species, or those that show a decrease in embryonic dry weight over development of 20%–40%, include the following *Poeciliopsis* species: *P. fasciata*, *P. monacha*, *P. infans*, *P. gracilis*, *P. hnilickai*, *P. catemaco*, *P. turrubarensis*, and *P. scarlii*. Low to moderate levels of placentotrophy range from a decrease in embryonic dry weight over development of ca. 15% to an increase of ca. 60% and are exhibited by the following *Poeciliopsis* species: *P. latidens*, *P. baenschi*, *P. lucida*, *P. occidentalis*, and *P. viriosa*. Finally, species with extensive placentotrophy have an increase in dry weight during development of ca. 500%–11,000% and include the following *Poeciliopsis* species: *P. prolifica*, *P. paucimaculata*, *P. elongata*, *P. presidionis*, *P. turneri*, and *P. retropinna* (Reznick et al. 2002b). Other independent cases of extensive placentotrophy are found in *H. formosa* (Turner 1940b; Scrimshaw 1944a; Schrader & Travis 2005; increase of ca. 4500%), *Poecilia branneri*, and *Poecilia bifurca* (Pires et al. 2010; increase of ca. 60,000%). Cases of less extensive placentotrophy are found in *Phalloceros caudimaculatus* (Arias & Reznick 2000; increase of ca. 100%) and *X. ctenolepis* (Reznick et al. 2007a; increase of ca. 400%).

Marsh-Matthews et al. (2001, 2005; Marsh-Matthews, **chapter 2**) and Marsh-Matthews and Deaton (2006) used radioactively labeled amino acids to show that *Gambusia geiseri* and *G. affinis* transfer some resources from the mother to the young during development, but both species are considered lecithotrophic based on the percent of weight lost by embryos during development. Trexler (1997) has shown that *Poecilia latipinna* can be either lecithotrophic or weakly placentotrophic, possibly in response to food availability. These observations suggest that species categorized as lecithotrophic based on the pattern of embryonic dry-weight change over development may still have some capacity to transfer nutrients from the mother to developing young.

3.3.2 Superfetation

Superfetation (also "superfoetation" or "superembryonation"; Veith 1979) is "the occurrence of more than one stage of developing embryos in the same animal at the same time" (Scrimshaw 1944b, 180); that is, it is the occurrence of fertilization and development of a new brood before the former brood is born (Turner 1937; see also Turner 1940c; Thibault 1974; Thibault & Schultz 1978). "Sequential brooding," used to describe multiple, ontogenetically staggered broods coexisting within some bivalves (Cooley & Foighil 2000), is synonymous with superfetation. The term "clutch overlap" (Burley 1980; Hill 1986; Travis et al. 1987), used to describe reproductive characteristics in some birds, is functionally similar to superfetation, but it encompasses simultaneous provisioning of different clutches before and after birth and does not apply to organisms in which maternal provisioning ends at parturition, such as poeciliids. "Litter overlap" was used by Downhower et al. (2002) to describe the simultaneous presence of yolking (or fully yolked) eggs and developing embryos within a female. They considered such overlap to be a form of superfetation. This is a common and interesting phenomenon in poeciliids but is not superfetation, because all developing embryos are part of a single brood and will be born at the same time, before the next clutch of eggs is fertilized.

There is pronounced variation in the number of broods present in the ovary among those species that have superfetation. The degree of superfetation (or a "superfetation index") can be expressed as either the average or the maximum number of developmentally distinct broods found simultaneously within females, depending on whether the goal is to characterize average or maximum reproductive output. All species in the genus *Poeciliopsis* are capable of superfetation (Turner 1937, 1940c; Scrimshaw 1944b; Thibault 1974; Thibault & Schultz 1978; Reznick & Pires, unpublished data), but the maximum number of simultaneous broods per female in a given species ranges from two (e.g., *P. monacha*) to five (e.g., *P. prolifica*). Females of *X. ctenolepis* have been found to carry up to six simultaneous developing broods (Reznick et al. 2007a), and *H. formosa* and *Poecilia branneri* may carry up to five simultaneous developing broods (Turner 1937,1940b, 1940c; Scrimshaw 1944b; Travis et al. 1987; Pires et al. 2010). Intraspecific variation in superfetation can be largely due to variation in female size and food availability (Travis et al. 1987; Pires et al. 2007; Banet & Reznick 2008). One study also documents differences among populations within a species

that appear to be a function of habitat (Zúñiga-Vega et al. 2007; see also section 3.5.1 below).

Reports from aquarists indicate the possible presence of superfetation in *Priapella bonita*, *Phalloptychus januarius*, *Poecilia* (*Micropoecilia*) *branneri* (Stoye 1935, in Turner 1937 and Scrimshaw 1944b), and *Priapichthys fria* (*Pseudopoecilia fria*; Turner 1940c; Henn 1916, in Turner 1937). Turner (1940c) listed additional, unconfirmed reports of superfetation in *Phallichthys fairweatheri* (*Dextripenis evides*), *Gambusia vittata* (*Flexipenis vittata*), and *Priapichthys chocoensis* (*Diphyacantha chocoensis*). Scrimshaw (1944b) described unconfirmed cases of superfetation in *Neoheterandria tridentiger* (*Allogambusia tridentiger*), *Brachyrhaphis cascajalensis*, *Brachyrhaphis episcopi*, *Gambusia nicaraguensis* (*Gambusia dovii*), *Gambusia holbrooki*, *Gambusia nobilis*, *Poecilia reticulata* (*Lebistes reticulatus*), and *Poecilia sphenops* (*Mollienesia sphenops*). He hypothesized that some individuals in all poeciliid species may express superfetation. Our personal observations on *Priapella bonita, Phallichthys fairweatheri, G. vittata, G. holbrooki, Brachyrhaphis episcopi, Poecilia reticulata, and Poecilia sphenops* have failed to confirm these earlier reports. From the species mentioned in these reports, we have been able to confirm superfetation only in *Neoheterandria tridentiger* (described in Stearns 1978), *Poecilia branneri, Phalloptychus januarius, Priapichthys fria*, and *Priapichthys chocoensis*.

It is clear that an exhaustive survey for the presence and level of true superfetation within poeciliids is still needed. In this process, it is critical that researchers demonstrate the ability to identify distinct developmental stages of embryos (including within-brood variation in embryo development) and abnormal embryos; runts and aborted or deformed embryos can be mistaken for ones that are in early stages of development. In addition, variation in the degree of egg yolking (i.e., prefertilization maternal provisioning) should be excluded from analysis of superfetation, for superfetation refers only to multiple broods of developing embryos. Ignoring any of these factors may lead to misleading reports of true superfetation, as for *Pamphorichthys hollandi* (Casatti et al. 2006a) and *G. hubbsi* (Downhower et al. 2002).

Our numerous observations of dissected reproductive individuals show that a hallmark of superfetation is the simultaneous presence of similar-sized broods in noncontiguous stages of development within a female. As the degree of superfetation increases, however, the researcher's ability to discern simultaneous broods in clearly noncontiguous stages decreases. Superfetation can still be unambiguously differentiated from large within-brood variation if distinct broods have similar numbers of embryos and if there is a stepped distribution of embryo size, which occurs in highly placentotrophic species. Large broods followed by contiguous broods with a significantly smaller number of embryos (as described in *Poecilia formosa*; Monaco et al. 1983) may represent aborted, regressing embryos rather than true superfetation (R. Riesch, pers. comm.). They may also represent within-brood variation in the stage of development. Such observations are worth recording, for they may reflect important physiological differences among species in their reproductive cycles and hence may represent the necessary variation for the evolution of true superfetation. The underlying biological causes of such variation as well as the extent to which this variation is regulated by environmental and genetic factors are all important questions still to be addressed.

3.3.3 The association between superfetation and placentotrophy

The work of Turner (1937, 1940c), Scrimshaw (1944a, 1944b), and Thibault and Schultz (1978) clearly implied a "parallel development" (Scrimshaw 1944b) between placentotrophy and superfetation. Thibault and Schultz (1978) then hypothesized that there is an adaptive value to the joined presence of both traits in stable environments and proposed that poeciliids could be grouped as either having placentotrophy and superfetation or lecithotrophy and the absence of superfetation. Reznick and Miles (1989a) later described a "nearly perfect" association between superfetation and placentotrophy, with only one species, *Poeciliopsis monacha*, known to be lecithotrophic with superfetation. Since then, a more thorough survey of the genus *Poeciliopsis* revealed that all lecithotrophic ($n = 8$) or incipient placentotrophic ($n = 5$) species are capable of having up to two or three simultaneous developing broods (Reznick et al. 2002b; Reznick and Pires, unpublished data). In addition, Arias and Reznick (2000) and Pires (2007) reported the presence of placentotrophy without superfetation in *Phalloceros caudimaculatus* and in the genus *Pamphorichthys*, respectively. It is thus now clear that superfetation and placentotrophy can evolve independently. However, the frequency with which both traits are found together and the joint expression of extensive superfetation and extensive placentotrophy in *H. formosa*, *Poeciliopsis* spp., and *X. ctenolepis* suggest that these traits are indeed correlated, but imperfectly so. The frequent association between superfetation and placentotrophy suggests that the evolution of one of these traits may facilitate the subsequent evolution of the other. The investigation of such a relationship, and of its significance, will contribute greatly to understanding the evolution of reproductive adaptations in poeciliids, as discussed in the remainder of this chapter.

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3.4 Evolutionary transitions in the Poeciliidae

Hrbek et al. (2007) present a well-resolved, DNA sequence–based phylogeny for the Poeciliidae that gives us some basis for making inferences about the evolution of livebearing, and variations on the theme of livebearing, in this clade. First, we will suggest an expected sequence of events, and then we will compare our suggestion with what we can infer from the combination of the phylogeny for these taxa and the distribution of life histories throughout the phylogeny.

Livebearing requires internal fertilization, but many egg layers have this capacity, so we assume that an egg-laying ancestor of the Poeciliidae first evolved internal fertilization, then viviparity; that is, the gonopodium and associated internal fertilization are shared, derived traits of the Poeciliidae. Lecithotrophy without superfetation represents the simplest form of viviparity. Turner (1940c) observed that the eggs of lecithotrophic poeciliids were no different in structure from egg layers in other taxa in the order Cyprinodontiformes, in which the family Poeciliidae is included, so it appears that little or no structural modifications in the egg were associated with this transition. We assume that superfetation and placentotrophy demand subsequent adaptations, such as those that allow for increased flexibility in the yolking and in the timing of egg fertilization and birth of young, as well as for a decrease in the amount of yolk that is provisioned before fertilization and for an increased ability to transfer nutrients after fertilization. Logic thus suggests that the sequence of events was, first, to evolve internal fertilization, then egg retention, then simple viviparity (lecithotrophy without superfetation), then either superfetation or placentotrophy. The distribution of these presumably more derived traits, as discussed in the previous section, suggests that either superfetation or placentotrophy can evolve by itself (Pollux et al. 2009). The question now is whether or not the distribution of these traits in the family provides evidence for such logical transitions.

Tomeurus gracilis is an egg-laying species with internal fertilization, thus exhibiting life-history characteristics that we might expect of a basal species in the family. Hrbek et al. (2007) instead found that *Tomeurus* is not the sister taxon to the remainder of the family; *X. ctenolepis* is. We (Reznick et al. 2007a) have recently confirmed the earlier observations of Hubbs (1950) that *Xenodexia* has both superfetation and placentotrophy. This unexpected distribution of life histories in the basal branches of the family tree suggests alternative hypotheses for the way life histories have evolved in this clade. One is that the common ancestor of the family had a life history that was most similar to *Tomeurus*. For this to be true, there must also have been
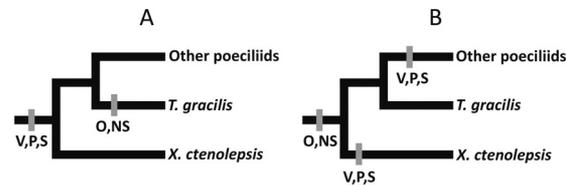

Figure 3.1   Competing hypotheses for the evolution of life-history traits in the basal poeciliid species (based on phylogeny from Hrbek et al. 2007). Hypothesis A requires the common ancestor of poeciliids to have viviparity (V), placentotrophy (P), and superfetation (S), which were lost in the ancestor of *Tomeurus gracilis*. In hypothesis B the common ancestor of poeciliids has oviparity (O) and nonsuperfetation (NS), thus requiring the origin of viviparity, placentotrophy, and superfetation in *Xenodexia* to be independent of the origin of the same traits in other poeciliids. Under both scenarios, placentotrophy and superfetation (but not viviparity) must have been lost independently many times by other poeciliids. *Phalloptychus januarius* and *caudimaculatus*, the two closest sister taxa to *Tomeurus* in the "other poeciliids" branch in the phylogeny from Hrbek et al. 2007, are both viviparous. *Phalloptychus januarius* exhibits superfetation; *Phalloceros caudimaculatus* does not.

the independent evolution of viviparity, superfetation, and placentotrophy in the ancestor of *Xenodexia*, then again throughout the remainder of the phylogeny. Alternatively, the common ancestor of the family may have been viviparous and may even have had superfetation and/or placentotrophy. For this to be true, these traits must have been lost in the ancestor of *Tomeurus* (fig. 3.1).

It is difficult to distinguish between these alternatives by looking at the poeciliids alone, but this task may be more feasible if we enlarge the analysis to include species from throughout the order Cyprinodontiformes. The sister clade to the family Poeciliidae includes *Oxyzygonectes*, *Jennynsia*, and *Anableps*. The latter two genera have internal fertilization, are viviparous, and are matrotrophic but lack superfetation. *Anableps* has a follicular placenta that is similar to that of some species in the genus *Poeciliopsis* (Turner 1938, 1940b). Development in *Jennynsia* is unlike that of *Anableps* or of any of the poeciliids. The eggs of *Jennynsia* are fertilized while retained in the follicle, but ovulation takes place after the initial development of segments; development is completed in the ovarian lumen (Turner 1940a). *Oxyzygonectes* is an egg layer with external fertilization. It appears that *Oxyzygonectes* is basal to this clade (Hrbek & Meyer 2003), so we are left with alternatives that are similar to those presented by the Poeciliidae: there may have been a common ancestor to both clades that was viviparous, with *Oxyzygonectes* representing a loss of viviparity, or viviparity and placentotrophy may have evolved independently in both clades. We are currently developing a molecular phylogeny that includes representatives from throughout the cyprinodont order with the goal of using it to develop a more highly resolved hypothesis for the evolution of life histories in these taxa. For now, the most likely life history of the common ancestor of the Poecili-

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idae and the pattern of evolution within the family remain unresolved.

3.5 Ecological hypotheses for the evolution of placentotrophy and superfetation

Despite the uncertainties related to the distribution of reproductive traits within Cyprinodontiformes and, more specifically, poeciliids, all results so far strongly suggest multiple, independent origins of superfetation and placentotrophy within the family. The adaptive significance of these traits, however, remains largely unknown. Some of the hypotheses that have been introduced in the literature to explain the evolution of matrotrophy and superfetation focus on the ecological conditions that may select for these traits. These generally fall into two categories: locomotor performance hypotheses and resource availability hypotheses (Pollux et al. 2009).

3.5.1 Locomotor performance hypotheses

The evolutionary transition from oviparity to viviparity implies an increase in the length of time that a female is physically bound to her developing offspring. Although the female is emancipated from a nest, she must still carry the developing offspring with her during daily activities, including foraging and predator avoidance. Studies in a variety of taxa have shown that egg retention and viviparity reduce locomotor performance (Shine 1980; Bauwens & Thoen 1981; van Damme et al. 1989; Plaut 2002; Ghalambor et al. 2004; Wu et al. 2004). Since matrotrophic species have a smaller initial egg size, the physical burden they carry for a given number of offspring, particularly at the early stages of pregnancy, is smaller than that of lecithotrophic species. Miller (1975) and Thibault and Schultz (1978) suggested that this resulted in a "streamlining" of matrotrophic species, thus reducing the locomotor costs of internal development. Further, they suggested that matrotrophy in concert with superfetation staggers the larger physical burden of later stages of development, amplifying the streamlining effect (fig. 3.2). Several lines of evidence add support to their hypothesis.

The size of the reproductive package a female carries has been linked to locomotor performance in the guppy, *Poecilia reticulata*. Guppies are lecithotrophic; their embryos lose dry mass over the course of development. However, guppy embryos have nearly a fourfold increase in wet mass between fertilization and birth because of an increase in the water content of developing embryos (Ghalambor et al. 2004). Ghalambor et al. (2004) examined different components of the escape response, or C-start (Weihs 1993),
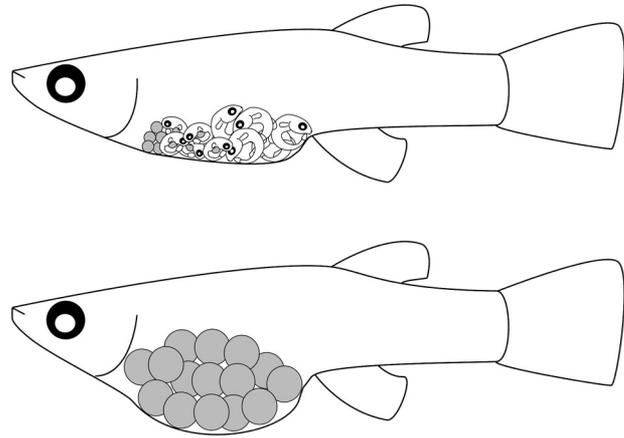

Figure 3.2   Illustration of hypothesized effects of superfetation and placentotrophy on body streamlining of poeciliid species. A lecithotrophic species without superfetation (below) must allocate all resources to offspring prior to fertilization. With superfetation, females are able to have an equivalent fecundity while allocating resources to embryos at different times. When superfetation is coupled with placentotrophy (above), not only is total resource allocation to offspring more spread out over time, but initial allocation is also considerably smaller. A possible functional consequence of this pattern of resource allocation is a more streamlined body, which may reduce the locomotor cost of viviparity.

in pregnant female guppies. They found that stage of pregnancy (and thus the size of the reproductive package) correlated well with maximum velocity, distance traveled, turning angle, and mean rotational velocity during an escape, with females at later stages of pregnancy, and thus with larger reproductive packages, showing impairment. They also found that guppies from high-predation localities, which have a higher reproductive allocation on average (e.g., Reznick et al. 1990; Reznick et al. 1997), performed better in many aspects of the escape response than low-predation guppies; however, they only did so when they were carrying embryos that were in earlier stages of development. High-predation guppies experienced a more rapid decline in velocity and distance traveled during the escape as the pregnancy progressed, suggesting a greater locomotor cost of reproduction for high-predation females due to the larger embryo size at later stages of reproduction.

Walker et al. (2005) verified the assumption that a faster escape response decreases the likelihood of predation during predator-prey interactions. They examined likelihood of predator evasion using the guppy and a natural predator, the pike cichlid *Crenicichla alta*, and found that predator evasion was positively correlated with two locomotor performance variables: rapid rotational velocity in the early stage of the escape response and "rapid tangential acceleration," a composite variable including net distance traveled, maximum velocity, and maximum acceleration. This result thus confirms that the reduced performance associated with pregnancy will increase susceptibility to predation.

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Locomotor costs of pregnancy are not limited to predator-prey interactions, however. Routine swimming, such as that used to maintain position against water flow velocity, can also be deleteriously affected during pregnancy. Studies of pregnant *G. affinis* revealed a significant decrease in critical swimming speed (Brett 1964) over the course of pregnancy (Plaut 2002). Body mass and cross-sectional area of the female increased as the pregnancy progressed. Surprisingly, the study did not find any change in tail beat amplitude or frequency when swimming at a fixed speed as the pregnancy progressed. Tail beat frequency and/or amplitude would be expected to increase if the decrease in critical swimming speed was due to the physical burden of pregnancy, which would result in increased drag and reduced flexibility, indicating that a female was working harder to swim at a fixed speed. Plaut (2002) suggests that the locomotor cost is thus caused by a physiological impairment rather than a physical burden during pregnancy.

Zúñiga-Vega et al. (2007) examined the relationship between superfetation and stream velocity in six inland and six coastal populations of the lecithotrophic *Poeciliopsis turrubarensis*. Because superfetation allows a female to carry multiple broods at different stages of development, the proportion of developing embryos that are in the largest stages of development at a given time can be decreased without reducing fecundity (fig. 3.2). Inland populations of *P. turrubarensis* live in areas with higher water velocity than coastal populations and thus are expected to have a higher cost of locomotion. The authors therefore predicted that inland populations would exhibit a higher degree of superfetation. Inland populations did have higher levels of superfetation, as well as larger brood sizes and a higher reproductive investment overall. The authors pointed out that superfetation allowed an increase in the rate of offspring production while bypassing the associated cost in increased physical burden.

Finally, a study of life-history traits in another fish family, the Zenarchopteridae, revealed that, in the genus *Dermongenys*, matrotrophic species produced fewer, larger offspring than lecithotrophs, while the trend was reversed in the genus *Nomorhampus*: matrotrophic species produced more, smaller offspring (Reznick et al. 2007b). The only consistent life-history correlate with matrotrophy was reproductive allocation, which was lower in matrotrophic species in both genera. The cost of locomotion was not directly addressed in this study, but a reduced cost of locomotion is implied in matrotrophic zenarchopterids if decreased reproductive allocation is correlated with increased locomotor performance.

The studies discussed above point to both matrotrophy and superfetation being consistently correlated with reduced reproductive allocation and, consequently, with a reduced burden of pregnancy via improved locomotor performance. It is noteworthy, however, that all evidence thus far is circumstantial; no study conducted to date has specifically addressed differences in locomotor abilities between matrotrophic and lecithotrophic species or between species with and without superfetation.

3.5.2 Resource availability hypotheses

Another subset of ecological hypotheses focuses on resource availability. Matrotrophic females start reproduction with small eggs and provide additional nutrient investment to offspring throughout development, allowing these females to spread investment into offspring over a greater period of time than strictly or predominantly lecithotrophic females can. Such differences in allocation patterns can be studied under the framework of life-history adaptations, as differences between capital and income breeders (Drent & Daan 1980; Houston et al. 2007), and this approach may hold the key to identifying a possible advantage of matrotrophy.

Trexler and DeAngelis (2003) developed a combination of analytical and simulation models to investigate what resource conditions would favor the matrotrophic reproductive mode over the lecithotrophic mode. The analytic model examines the reproductive success of each reproductive mode during a single reproductive event, assuming a size-number trade-off in initial egg number. For simplicity, it also assumes that terminal offspring size is the same for both reproductive strategies. From a set amount of starting resources, a lecithotroph produces fewer, fully yolked eggs. Since the matrotroph starts with a smaller egg, she can produce more eggs initially, which will then need additional resource investment throughout gestation. If resources are consistently abundant during the gestation period, the matrotroph thus has the potential to produce a greater brood size than the lecithotroph. However, if food levels are low or unpredictable, producing a large number of eggs may be counterproductive for the matrotrophic female because she risks having insufficient resources to nourish all offspring and may thus lose the entire brood. This leads to an important assumption in the model: when resources become scarce, the matrotroph is assumed to have the ability to abort and resorb energy from some offspring within the brood. The simulation model expands on the analytical model by examining lifetime reproductive success of each reproductive mode across a range of resource levels and embryo resorption capabilities. It takes growth, storage, and schedule of reproduction into account. Overall, the model suggests that matrotrophy is most likely to evolve in habitats where abundant resources are consistently available. The ability to abort and resorb offspring expands the

conditions under which a matrotroph would have higher lifetime reproductive success: without the ability to abort, the predicted habitat range for species with a matrotrophic reproductive mode is narrowed to only areas with the highest, most predictable resource levels.

Recent empirical studies do not support the assumption that matrotrophic species abort offspring in low food conditions. Marsh-Matthews and Deaton (2006; Marsh-Matthews, **chapter 2**) examined the effect of food level on the reproduction of *G. geiseri*. This species is primarily lecithotrophic, but it has been found to allocate small amounts of nutrients to embryos after fertilization, particularly under high resource levels (Marsh-Matthews & Deaton 2006). Although the authors did find indications of abortion, it was independent of food level: both high- and low-food treatments showed similar frequencies of aborted embryos. Banet and Reznick (2008), using closely related placentotrophic and lecithotrophic species from the genus *Poeciliopsis*, found no evidence that placentotrophic species abort offspring in low food conditions. Instead, they found that when food level was reduced after fertilization, placentotrophic females produced smaller offspring and sacrificed body condition to maintain all embryos in a developing brood.

Similarly to placentotrophy, the other facet of reproductive mode variation in poeciliids, superfetation, also allows a female to stagger periods of heightened investment over time without reducing fecundity. Like the evolution of matrotrophy, then, the evolution of superfetation may also be studied in the context of varying resource allocation strategies. For a set rate of offspring production in a lecithotroph, superfetation decreases resource investment in each brood due to a reduction in brood size but increases the frequency at which broods are initiated. In a matrotroph with superfetation, a female spreads resource investment over the course of gestation. In species with MI > 1, the dry mass of embryos increases nonlinearly during development (e.g., Reznick et al. 2007a, for *X. ctenolepis*) in such a way that maternal investment progressively increases as development occurs. Superfetation allows the matrotroph to space out these periods of increased investment, so that fewer offspring are at the growth phase of development at a given time. The Trexler-DeAngelis model is currently being modified to take into account these studies and to include superfetation (J. C. Trexler, pers. comm.).

3.6 Summary and future research

The adaptive significance of matrotrophic reproduction remains one of the least studied aspects of life-history evolu-

tion. The extensive variation on the theme of livebearing exhibited by poeciliid fishes makes them excellent model organisms for studies aiming to address different aspects of the evolution of matrotrophy and, more specifically, placentotrophy. As described throughout the chapter, the past 20 years have seen some progress in the first three study directions proposed by Reznick and Miles (1989a) for such investigations. In summary:

1. Life-history descriptions of key species have revealed the independent origins of placentotrophy and superfetation (e.g., *X. ctenolepis*, Reznick et al. 2007a; the subgenus *Micropoecilia*, Pires et al. 2010; all species in the genus *Poeciliopsis*, Reznick et al. 2002b; Reznick and Pires, unpublished data). In addition, interpopulation comparisons have started to identify the environmental and genetic components of within-species variation in reproductive mode (e.g., Schrader & Travis 2005; Pires et al. 2007), thus providing raw material for investigations of life-history evolution (Reznick & Travis 1996).
2. The descriptive work of life histories within the genus *Poeciliopsis*, coupled with its phylogenetic study (Reznick et al. 2002b) and recent theoretical advances (Trexler & DeAngelis 2003), has made it possible to compare pairs of sister species with contrasting modes of reproduction in common garden conditions within the context of a hypothetico-deductive framework (Banet & Reznick 2008).
3. Phylogenetic studies (e.g., Breden et al. 1999; Ghedotti 2000; Mateos et al. 2002; Lucinda 2005a, 2005b; Hrbek et al. 2007; Meredith et al. 2010) have focused on different taxonomic levels and have started to provide a much needed basis upon which inferences of evolutionary trends within poeciliids can be drawn.

The current knowledge concerning variation and patterns of evolution in life-history traits and reproductive mode in the family Poeciliidae provides a good foundation for understanding selective factors for the evolution of these traits. However, there is still much work to be done; the adaptive significance of placentotrophy, in particular, is still largely unknown. We suggest that the research agenda described above be further explored; in addition, this agenda should be expanded to address the following two general areas:

1. The examination of the relationship among life-history traits. Are there sets of integrated traits that give us clues to understand the observed patterns of evolution?

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If so, what are the functional and physiological conse-quences of the combination of such traits?

2. Experimental studies examining current hypotheses for the evolution of the placenta.

The development of more comprehensive phylogenetic reconstruction of relationships within poeciliids and be-tween the family and its sister groups will allow us to test specific predictions derived from these hypotheses within a phylogenetic, comparative context. Multiple independent origins of placentation within the family make it an excel-lent group to conduct comparative studies to test current models of life-history evolution. Common garden studies focusing on life-history variation within the family and large-scale comparative analyses will certainly contribute insights into the questions of how and why placentotro-phy, superfetation, and their correlated life-history traits evolved in poeciliids.

Acknowledgments

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