

Independent Origins and Rapid Evolution of the Placenta in the Fish Genus *Poeciliopsis*

David N. Reznick,^{1*} Mariana Mateos,^{2†} Mark S. Springer¹

The evolution of complex organs is a source of controversy because they require the contributions of many adaptations to function properly. We argue that placentas are complex, that they have evolved multiple times in *Poeciliopsis*, and that there are closely related sister taxa that have either no placentas or intermediate stages in the evolution of a placenta. Furthermore, placentas can evolve in 750,000 years or less, on the same time scale as suggested by theoretical calculations for the evolution of complex eyes. Independent origins of such complexity, accompanied by sister taxa that either lack or have intermediate stages in the evolution of the trait, present an opportunity to study the evolution of novelty and complexity from a comparative, evolutionary perspective.

Darwin addressed the conceptual difficulty of complex adaptations, or “organs of extreme perfection,” with his treatment of the evolution of the eye: “To suppose that the eye with all its inimitable contrivances for adjusting the focus to different distances, for admitting different amounts of light, and for the correction of spherical and chromatic aberration, could have been formed by natural selection, seems, I freely confess, absurd to the highest degree” (1, p. 155). Said differently, the dilemma posed by a complex adaptation is that it requires many independent elements, all of which must be present for the organ to be useful. If so much is required for an eye to function properly, then how could it evolve? Darwin proposed that complex eyes could have been formed with a succession of photosensitive organs, each a bit more complex than its predecessor and each favored by natural selection because of the advantage that accrued to the possessor. Visualizing such a process would be easiest if steps in this sequence were preserved in closely related living organisms; however, no such sequence exists for eyes because the intermediate stages have been lost through extinction. Our best alternative is to compare distantly related species that display what appear to be intermediate stages in evolution, as was done by Darwin with examples from across the mollusk or arthropod phyla, or to approach the problem from a theoretical perspective (2).

Placental reproduction is like the eye both in

the complexity of the adaptation and because it has evolved repeatedly in different groups of organisms, including fish, amphibians, reptiles, and mammals (3). The mammalian placenta has been far better characterized than that of any other placental organism and serves as a model for the complexity of the adaptation. Rossant and Cross (4) summarize results on 50 loci that regulate the development of the placenta. Additional adaptations include loci that are members of gene families that produce protein hor-

mones and hemoglobin but are only expressed in the fetus and are specifically adapted to fetal development (5, 6). Other adaptations include facilitation of gas exchange, transfer of nutrients, disposal of waste products, and suppression of immunological interactions or other forms of intergenomic conflict between the mother and embryo (5). All live-bearing organisms with extensive maternal provisioning after fertilization face similar demands and must have similar adaptations.

Fish in the genus *Poeciliopsis* (Poeciliidae) display variation in live-bearing that ranges from species that retain eggs after fertilization with no further maternal provisioning (lecithotrophy or yolk feeding) to those that have variable amounts of maternal provisioning after fertilization (matrotrophy or mother feeding) (7). Turner (8) found that high levels of post-fertilization provisioning are associated with an elaboration of maternal and fetal tissues that he named the “follicular pseudoplacenta” in recognition of their apparent functional similarity to a mammalian placenta. Furthermore, he found a positive association between the amount of maternal provisioning provided before versus after fertilization and the elaboration of structures that form the interface between mothers and developing young. There thus appears to be variation from species that have no such specialized tissues to those with highly elaborate placentas, plus a range of species in between with less elaborate placentas.

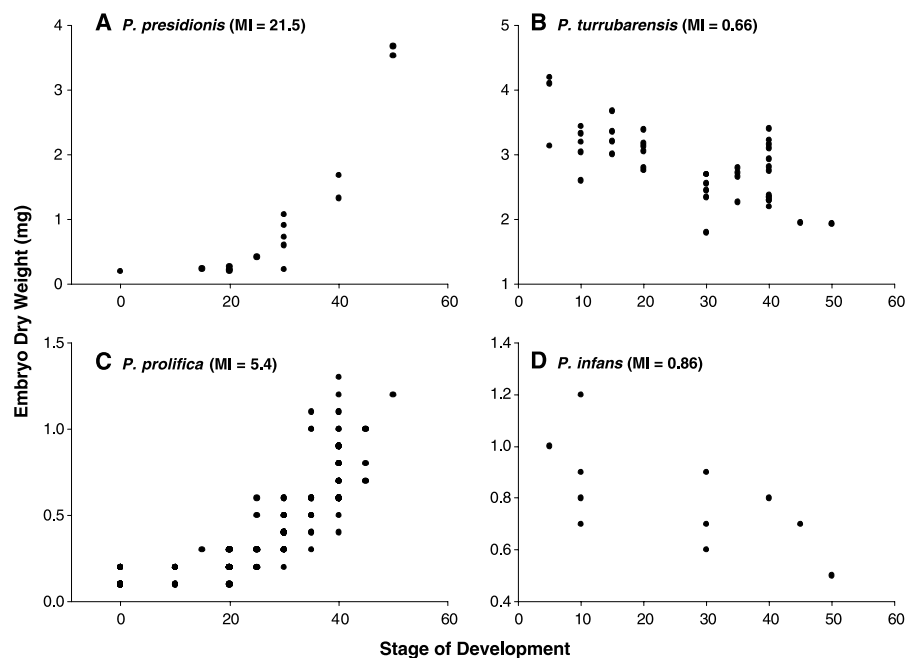


Fig. 1. Sample scatter plots of mean embryo dry weight (mg) versus stage of development for four species of *Poeciliopsis*. (A) *P. presidionis*, a highly matrotrophic species from the “mostly southern” clade. (B) *P. turrubarensis*, a lecithotrophic species from the “mostly southern” clade. (C) *P. prolifica*, a highly matrotrophic species from the “mostly northern” clade. (D) *P. infans*, a lecithotrophic species from the “mostly northern” clade. The values on the x axis represent progressive stages of development, ranging from early eye pigment (stage 10) to fully developed embryos that were ready to be born (stage 50), according to the methods described in (10) and (22).

¹Department of Biology and Center for Conservation Biology, University of California, Riverside, CA 92521, USA. ²Monterey Bay Aquarium Research Institute, 7700 Sandholdt Road, Moss Landing, CA 95039, USA.

*To whom correspondence should be addressed. E-mail: david.reznick@ucr.edu

†Present address: Department of Ecology and Evolutionary Biology, Biosciences West Room 310, University of Arizona, Tucson, AZ 85721, USA.

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We quantify maternal provisioning with the “matrotrophy index” (MI), which is the estimated dry mass of offspring at birth divided by the dry mass of the egg at fertilization (9, 10) (table S1). Lecithotrophic species have MI values near 0.6 to 0.7, meaning that they lose mass during development because of the costs of metabolism; similar mass losses are observed in egg-laying species of fish (9). Matrotrophic

species have values that range from near 1, implying some post-fertilization provisioning to offset the costs of development, to more than 100, indicating extensive post-fertilization maternal provisioning (Fig. 1).

The MI values for all species in this genus and their frequency distribution (Figs. 2 and 3) indicate that some species have little or no maternal provisioning (MI = 0.6 to 0.7),

moderate amounts of maternal provisioning ($0.8 < MI < 2$), or extensive maternal provisioning ($MI > 5$) (10). We found no MI values between 2 and 5.

We chose an MI value of 0.7 as a benchmark for evaluating statistically whether or not there was maternal provisioning after fertilization. By this criterion, many species with $MI > 0.8$ were significantly greater than 0.7 (Fig. 3). Conversion efficiencies may well vary among species and be influenced by environmental factors. Populations may also vary in post-fertilization provisioning. Therefore, one cannot interpret the MI value as an absolute measure of maternal provisioning for a species; however, the distribution of values for the genus indicates that there is a continuum of variation from species that have no maternal provisioning after fertilization to those with moderate amounts of provisioning. There is then a discontinuous leap to those that have extensive provisioning. Species with extensive matrotrophy have very little yolk deposition before fertilization, then an exponential increase in mass through development, similar to the pattern of embryonic growth of mammals (Fig. 1) (11). There are thus gradations in the degree to which the mothers of different species provision young during development.

A molecular phylogeny (12) (Fig. 3) reveals that the genus bifurcates into two clades that correspond to what had originally been classified as two genera or subgenera (*Aulophallus* and *Poeciliopsis*) (13). The *Poeciliopsis* clade in turn subdivides into “mostly southern” and “mostly northern” subclades (12). Matrotrophy indices above 5 (Fig. 3) occur in species in the subgenus *Aulophallus*, in *P. prolifica* from the “mostly northern” clade, and in *P. turneri* and *P. presidionis* from the “mostly southern” clade. The distribution of taxa with substantial maternal provisioning in three distinct groups, as opposed to one or two, is supported by high bootstrap percentages over most of the mitochondrial phylogeny (12) (Fig. 3) and by Swoford-Olsen-Waddell-Hillis tests (10, 14). *P. prolifica*, *P. turneri*, and *P. presidionis* are all nested within clades with sister taxa that have no maternal provisioning ($MI = 0.6$ to 0.7) and small to moderate amounts of maternal provisioning ($MI = 0.8$ to 2). The most parsimonious hypothesis is that the common ancestor of the genus lacked maternal provisioning, so that there have been three independent origins of extensive maternal provisioning within the genus (10) (Fig. 3 and figs. S1 and S2).

Theoretical calculations suggest that complex eyes could have evolved rapidly, possibly in 400,000 years (2). However, inadequate taxon sampling and the absence of fossil intermediates preclude accurate empirical quantification of the time scale over which adaptations such as the vertebrate eye and the mammalian placenta evolved. In contrast, taxonomic diversity within *Poeciliopsis*

Fig. 2. Frequency histogram of MI values for all species in the genus *Poeciliopsis*. MI values are untransformed but are plotted on a natural logarithm-transformed x axis. A value of 1 corresponds to embryos that maintain a constant dry mass throughout development and hence receive sufficient maternal resources to offset the costs of metabolism and growth. Species coded in blue have MI values that do not differ significantly from 0.7. Those coded in green have MI values that are significantly greater than 0.7 but less than 5. Those coded in red have MI values that are greater than 5 (10) (table S1). There is an overlap in the blue and green categories because of differences in sample size and within-population variation in offspring size, and hence in whether or not a given MI value is significantly greater than 0.7.

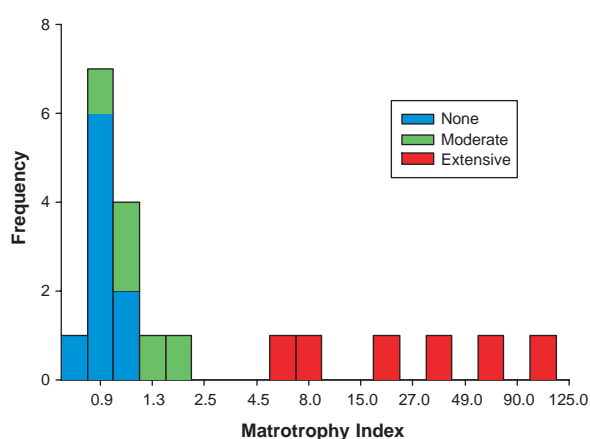
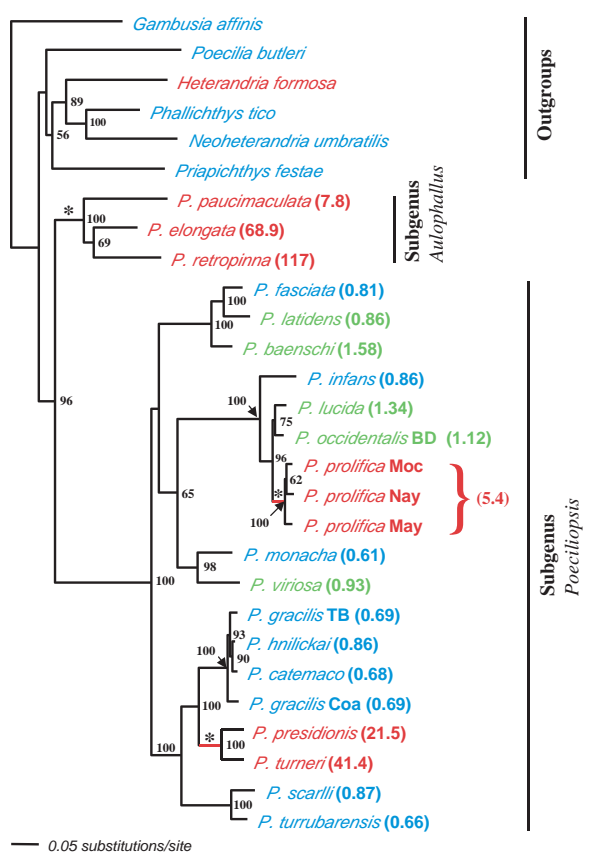


Fig. 3. Maximum likelihood phylogram (GTR + Γ + I model; $-\ln L = 20791.541$, where GTR is General Time Reversible, Γ is a gamma distribution of rates, and I is the proportion of invariant sites) for the genus *Poeciliopsis*, based on mitochondrial gene sequences (cytochrome b, nicotinamide adenine dinucleotide, reduced form, subunit 2) from Mateos *et al.* (12). Bootstrap values for clades supported above the 50% level are shown adjacent to branches. MI values for all taxa, not including outgroups, are included in parentheses next to each species. The results of statistical comparisons between the observed slope and the slope expected if $MI = 0.7$ are also shown (color coding). The significance of the observed MI was based on a *t* test [$t(0.7)$] that compared the slope of the regression of logarithmically transformed embryo weight on the stage of development with the slope that would be expected if 30% of the initial dry mass were lost during development. ($MI = 0.7$) (23). Each species was then coded as either having no evidence of post-fertilization maternal provisioning (blue), moderate amounts of maternal provisioning (green; MI range from 0.86 to 1.58), or extensive maternal provisioning ($MI > 5$). Species coded in red or green have MI values that are significantly greater than 0.7. The red branches associated with *P. prolifica*, *P. presidionis*, and *P. turneri* are those associated with estimates of the rate of evolution of extensive maternal provisioning. Asterisks denote branches on which extensive maternal provisioning evolved in the genus *Poeciliopsis*.



captures the evolution of a complex adaptation on a much finer scale. We used a relaxed molecular clock approach (10, 15, 16) to estimate the time required for extensive maternal provisioning to evolve in *P. turneri*, *P. presidionis*, and *P. prolifica*. Our point estimates are 2.36 and 0.75 million years ago for the “mostly southern” and “mostly northern” species, respectively (10). These time intervals estimate the maximum time available for the evolution of extensive maternal provisioning. The actual time is less than or equal to these intervals. It is thus clear that complex adaptations can evolve rapidly, on the same scale as theorized by Nilsson and Pelger (2) for the evolution of eyes.

The more important feature of these results is the scope of variation that is present within a group of closely related organisms and hence the potential for using these fish to study the evolution of novelty and complexity. Given the likely complexity of the adaptations associated with extensive maternal provisioning, this range of variation in *Poeciliopsis* is comparable to finding a single genus that has three independent origins of elaborate eyes, including congeners that have either no eyes or eyes in various intermediate stages of evolution.

The availability of three separate clusters of extensive maternal provisioning and the ability of some of these species to hybridize with one another (17, 18) provide the necessary raw material for evaluating the repeatability of the underlying mechanisms that generate this adaptation. For example, the subgenus *Poeciliopsis* includes the species that hybridize in nature to produce hybridogenetic and gynogenetic clones. Additional hybrids have been produced artificially (17, 18). A compelling feature of these hybridizations is that the female partner is always *P. monacha* (19), which has no post-fertilization maternal provisioning (Fig. 3) and hence is insulated from many aspects of intergenomic conflict. Furthermore, there are other species scattered throughout the family Poeciliidae that have either moderate or extensive amounts of maternal provisioning (20, 21). Although phylogenetic relationships among the rest of the family have yet to be resolved in comparable detail to the genus *Poeciliopsis*, there clearly are additional opportunities to evaluate independent origins of placentas. The extensive literature on placental reproduction in mammals provides guidance for the kind of adaptations one might find when evaluating a similar adaptation in fish. Experimentally accessible phenomena in Poeciliidae might include the progressive development of the genetic and morphological mechanisms associated with maternal provisioning or the escalating intergenomic conflict associated first with egg retention and then with the increasing commitment of maternal re-

sources after fertilization. These adaptations cannot be directly studied or manipulated in placental mammals, because their placentas are derived from a common ancestor that lived more than 100 million years ago and because the intermediate stages associated with this adaptation have long since been lost through extinction.

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Supporting Online Material

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 Materials and Methods
 Supporting Text
 Figs. S1 and S2
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Waves of Larch Budmoth Outbreaks in the European Alps

Ottar N. Bjørnstad,^{1*} Mikko Peltonen,² Andrew M. Liebhold,² Werner Baltensweiler³

Spatially extended population models predict complex spatiotemporal patterns, such as spiral waves and spatial chaos, as a result of the reaction-diffusion dynamics that arise from trophic interactions. However, examples of such patterns in ecological systems are scarce. We develop a quantitative technique to demonstrate the existence of waves in Central European larch budmoth (*Zeiraphera diniana* Gn.) outbreaks. We show that these waves travel toward the northeast-east at 210 kilometers per year. A theoretical model involving a moth-enemy interaction predicts directional waves, but only if dispersal is directionally biased or habitat productivity varies across the landscape. Our study confirms that nonlinear ecological interactions can lead to complex spatial dynamics at a regional scale.

There is growing evidence that animal population dynamics are governed by nonlinear processes capable of producing a variety of

temporal patterns, including equilibrium dynamics, regular oscillations, and even chaotic dynamics (1, 2). When the corresponding nonlinear population models are embedded in spatial landscapes, they often predict a range of complex (“self-organized”) spatial dynamics varying from perfect synchrony (“nonlinear phase-locking”) to static “crystal lattices,” spiral waves, and “spatial chaos” (3–6). Although there has been considerable success in confirming the existence of these emergent

¹Departments of Entomology and Biology, 501 ASI Building, Pennsylvania State University, University Park, PA 16802, USA. ²USDA Forest Service, 180 Canfield Street, Morgantown, WV 26505, USA. ³Blumenbergstrasse 9, CH-8634 Hombrechtikon, Switzerland.

*To whom correspondence should be addressed. E-mail: onb1@psu.edu