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# Life history of *Xenodexia ctenolepis*: implications for life history evolution in the family Poeciliidae

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*Xenodexia ctenolepis* (Hubbs, 1950) is a uniquely asymmetrical species in the fish family Poeciliidae that is endemic to a remote region of Guatemala. In the present study, we describe its life history based on the dissection of 65 adult females from three different collections. We show that it is a livebearer, has superfetation, or the ability to carry multiple litters of young in different stages of development, and has matrotrophy, or placentation, which results in the dry mass of young at birth being three- to four-fold greater than the egg at fertilization. The size distribution of males is non-normal in a fashion that suggests a genetic polymorphism for age and size at maturity. Most phylogenies place *Tomeurus gracilis* as the sister taxon to the remaining members of the family Poeciliidae. Because *Tomeurus* is the sole egg-layer in the family, egg-laying is thought to represent the life history of the common ancestor. Because *Xenodexia* possesses three supposed derived traits (livebearing, superfetation and matrotrophy), this phylogenetic hypothesis suggests that *Xenodexia* has a highly derived life history with respect to other members of the family. By contrast, the most recent DNA-based phylogeny suggests *Xenodexia* is the sister taxon to the remainder of the family. If this proves to be true, it suggests that some or all of these life history traits may have been characteristic of the common ancestor to the family, then lost and re-evolved multiple times within the family.  *Biological Journal of the Linnean Society*, 2007, **92**, 77–85.

ADDITIONAL KEYWORDS: life history evolution – matrotrophy – placentation – superfetation.

## INTRODUCTION

*Xenodexia ctenolepis* was originally classified as its own subfamily in the family Poeciliidae by Hubbs (1950) and Rosen & Bailey (1963) based on its unusual morphology. One feature, which Hubbs described as unique among the fishes, is the asymmetry of the pectoral fins in mature males. The left pectoral fin is normal in appearance whereas the right pectoral fin is described as a 'clasper' with '. . . an assortment of hooks, pads, and other processes'. The dextral asymmetry of the pectoral fins is matched with a dextrally asymmetrical gonopodium, or the anal fin, which is modified as an intromittent organ in all male poeciliids. The mating behaviour of this fish has never been observed, and the function of these asymmetries remains unknown. Other distinguishing features include osteological features of the skull and gonopodial suspensorium (Rosen & Bailey, 1963). Parenti (1981) reclassified the Poeciliidae as the subfamily Poeciliinae and placed *X. ctenolepis* into its own tribe, but retained it in essentially the same unique status relative to the rest of the family. A recent revision of the Poeciliidae (Lucinda & Reis, 2005), which is based on an anatomical analysis, instead places *Xenodexia* in the tribe Poeciliini and as the sister taxon to *Poecilia* (Rosen & Bailey, 1963). By contrast, the most recent DNA-based phylogeny (Hrbek, Seckinger & Meyer, 2007) places *Xenodexia* as the sister taxon to the remaining members of the family. Because of unresolved discrepancies between available morphological (Parenti, 1981; Lucinda & Reis, 2005) and DNA-based phylogenies (Hrbek *et al*., 2007; Meyer & Lydeard, 1993) for the relationship between this taxa and the

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Cyprinodontiformes, we will refer to it as the family Poeciliidae, *sensu* Rosen & Bailey (1963).

Another unusual feature of *Xenodexia*, which it shares with the genus *Poeciliopsis*, is the presence of superfetation, or the ability of females to carry multiple broods of young in different stages of development. This aspect of *Xenodexia's* biology was described in the dissection by Hubbs (1950) of the only mature female collected by L. C. Stuart when he made the type collection of the species in 1931. Because *Xenodexia* resembles *Poeciliopsis* in having superfetation and in the structure of the gonopodium, Hubbs (1950) suggested that it may have ultimately been derived from that genus; however, Rosen & Bailey (1963) argued that its unique asymmetry and osteological features instead suggest that the resemblance to *Poeciliopsis* is superficial and that *Xenodexia* is distantly related to the rest of the family. All subsequent revisions have agreed with Rosen and Bailey.

Our complete knowledge of this species' life history is based on this single mature female. Because this fish has a restricted range in remote parts of the Rio Negro drainage of Guatemala, it has rarely been collected and has never become the subject of studies that extended beyond its systematic position, save for the one dissection by Hubbs (1950). The present study aimed to provide a more detailed description of the life history, based on the study of larger collections made after L. C. Stuart's original discovery and to discuss the implications of its life history for patterns of life history evolution within the family.

While the single dissection of Hubbs (1950) is sufficient to conclude that the species is almost certainly capable of superfetation, there remains much to be learned about its reproductive biology. In the present study, we present the analysis of dissections of over 60 females from three later collections of *Xenodexia*. We describe the degree to which development is fuelled by yolk vs. maternal resources provided during development, the number of young per litter, the number of litters normally carried by each female, and offspring size at birth. Because many of these variables can be correlated with female size, we also describe them in terms of this size-relationship, as well as defining the size at which females begin to reproduce. Some collections were sufficiently large to enable us to describe the size distribution of mature males.

The combined information on the life history and systematic status of *Xenodexia* is of interest because of the diversity of life histories seen in the family Poeciliidae (Rosen & Bailey, 1963). All species in the family are united by the possession of a gonopodium and associated gonopodial suspensorium and internal fertilization (Rosen & Bailey, 1963). All three revisions of the systematics of this group of fishes (Rosen & Bailey, 1963; Parenti, 1981; Lucinda & Reis, 2005) place *Tomeurus gracilis*, the sole egg-layer, as the sister taxon to the remainder of the family, which may suggest that this form of reproduction is ancestral to the rest of the family. If the *Tomeurus* life history is that of the common ancestor to the remainder of the family, then three major life history innovations evolved within the family (i.e. livebearing, superfetation, and postfertilization maternal provisioning). All other species are livebearing, and this systematic arrangement suggests that the common ancestor to the rest of the family was a livebearer. Most species are lecithotrophic, which means that eggs are fully provisioned prior to fertilization (Reznick & Miles, 1989). Some species have superfetation (Turner, 1940b; Scrimshaw, 1944) and some have matrotrophy, or postfertilization maternal provisioning. Matrotrophy is accompanied by elaborations of maternal and embryonic tissues that are the functional equivalent of a mammalian placenta (Turner, 1940a). The combination of life history descriptions with phylogenies suggest that superfetation and placentas have evolved repeatedly in the family (Reznick & Miles, 1989), although a complete description of the phylogeny of the family and associated distribution of life histories within the family is not yet available. *Xenodexia*'s apparent possession of what seems to be a derived life history relative to the common ancestor of the Poeciliidae suggests that it could play an important role in the reconstruction of the patterns of life history evolution in this taxa. For example, if it, rather than *Tomeurus*, proves to be sister to the remainder of the family, then it raises the possibility that the common ancestor to the family was not an egg layer and that egg-laying in *Tomeurus* represents a loss of livebearing, rather than the ancestral life history for the Poeciliidae. Alternatively, *Xenodexia* may represent an additional, independent origin of a derived life history within the Poeciliidae.

## MATERIAL AND METHODS

One goal was to generate a description of the life history of this species for eventual inclusion in a comparative study of patterns of life history evolution in the family. The key variables include the minimum and mean size of reproducing females, whether or not superfetation is present, and the characteristic number of developing litters if it is, whether or not eggs are fully provisioned prior to fertilization (lecithotrophy) and, if not, the magnitude of postfertilization provisioning (matrotrophy). We also estimated offspring size at birth, and litter size, generally as a function of female size because the two tend to be positively correlated. For males, we considered the average size of mature individuals and the nature of the mature male size distribution. The latter variable was of interest

because some species in the family Poeciliidae have bi- or polymodal or highly skewed size distributions of males. Because male growth slows or stops at maturity, such variation can reflect variation in the age at maturity. In some of these cases, the non-normal distributions have been shown to be the product of genetic polymorphisms for the age and size at maturity (Kallman, 1989). All of these features can be described from the study of large, preserved samples of fish.

### Collections

We collected data on female life histories by removing ovaries from a subset of the mature females in three large museum collections. The source of our material and number of individuals were: Collection 1: AMNH 24569 ($N = 19$), 3/19/1963, Collection 2: UMMZ 193928 ($N = 26$), 3/29/1973 and Collection 3: AMNH 32137 ($N = 20$), 3/29/1973. All collections were made with seines and rotenone. The collection sites were described as 'large jungle streams' with clear water, gravel/rubble substrates, water temperatures that ranged from 22–26 °C and moderate to torrential currents. All fish were initially preserved in formalin, then transferred to alcohol for long-term storage. Collection 1 was from Rio Xalbal, whereas collections 2 and 3 were from Rio Ixcan, both of which are tributaries of the Rio Negro. The Rio Negro is in turn within the Rio Usumacinta basin, which drains into the Gulf of Mexico.

### Generation of life history descriptions

We first measured the standard length of all females with digital calipers that report length to 0.01 mm as the distance from the tip of the lower jaw to the outer margin of the hypleural plate. Ovaries were extracted from a mid-ventral incision that extended from the gonapore to beneath the pectoral fins, then a second incision on the right side that extended vertically from the gonapore. It was then possible to lift a flap that exposed the ovary so that we could remove it without disturbing the other internal organs. The ovary was opened, all embryos were removed and were separated into distinct litters based on their stage of development.

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

Males were classified as mature on the basis of the complete metamorphosis of the anal fin, which can be recognized by the convergence of the third through fifth rays to form a distinct tip and the disappearance of what appears to be a sheath or cuticle that covers the gonopodium of immature individuals, so that the fin changes from being translucent to transparent. All immature males were staged, following the classification developed by Kallman & Schreibman (1973) to describe the metamorphosis of the anal fin in species in the genus *Xiphophorus*. Standard length was measured in the same fashion as females.

### Statistics

Female reproduction was characterized with the size range of reproducing females, the number of distinct developing litters found in each female, the number of offspring per litter, the total number of offspring, the dry mass of offspring in each litter, and the total dry mass of all developing offspring. The relationships between fecundity, offspring size, and mass of reproductive tissues within a locality were characterized as a function of female size with linear regression (SAS Institute, 1999). Comparisons among the three collections were made with analyses of covariance, using female length as a covariate (SAS Institute, 1999). We first evaluated whether or not there was significant slope heterogeneity among collections for the female length-dependent variable regressions. If the interaction between the covariate and collection was not significant, then we excluded the interaction and hence assumed that these slope were homogenous and tested for differences among collections in intercepts. We compared the means for individual localities by using a Bonferroni adjustment for multiple comparisons among means (available as part of the LSMeans Statement in the GLM procedure of SAS; SAS Institute, 1999).

We evaluated the pattern of maternal provisioning by analysing the pattern of change in the dry mass of the embryos during development. Lecithotrophic species fully provision eggs prior to fertilization, so the costs of metabolism during development are met with reserves present in the eggs prior to fertilization. As a consequence, embryos lose in the vicinity of 35% of their dry mass during development. If there is

maternal provisioning after fertilization, then embryos may maintain dry mass or even gain considerable amounts of mass between fertilization and birth. The stages of development were converted into numerical values ranging from 0 (eggs ready to be fertilized) to 50 (advanced embryos ready to be born) for the purposes of curve fitting. These values were first used to describe the patterns of weight loss in *Gambusia affinis* (Reznick, 1981) and *Poecilia reticulata* (Reznick & Endler, 1982) and were chosen to generate a straight line relationship between stage and dry mass for purposes of statistical comparisons of offspring size among localities using stage of development as a covariate. We retained the same numerical values for these analyses to make them comparable to our earlier work.

We evaluated the statistical relationship between the dry mass of individual embryos and the stage of development with a mixed-effects model (i.e. a model containing both random and fixed effects) in S-Plus (S-Plus, 2001). The basic model comprised a polynomial in which one or more of the coefficients were considered as random effects; for a discussion of such models, see Pinheiro & Bates (2000). Such models are important in the present analysis because multiple measures were taken on females in the form of multiple litters of developing young. The mixed-model approach allows for variation among females and the potential non-independence of litters within a female. We entered collection as a fixed effect and considered one or more of the coefficients (see below) as random effects. Different models were compared using a log-likelihood ratio test. Our first model initially ignored the possible contribution of individual and used stepwise regression to locate the best model, starting with a sixth-order polynomial including interactions. This initial analysis located an adequate descriptor of the relationship between the response variable, embryo weight, and the predictors, collection and stage. We then used a mixed model approach to account for possible effects due to individual female.

For the purposes of making comparisons among populations and species, we summarize the results of the statistical description of weight change during development with the 'matrotrophy index' (MI), which equals the estimated dry mass of the embryo at birth divided by the dry mass of the egg at fertilization. Lecithotrophic species thus have MI values in the vicinity of 0.6–0.7 because the embryo at birth tends to be approximately 60–70% of the dry mass of the egg at fertilization. Species with the proportionately highest levels of postfertilization maternal provisioning can have MI values of greater than 100, meaning that the dry mass of the young at birth is over 100-fold greater than the mass of the egg at fertilization. This index will systematically underestimate the extent to which mothers provision developing embryos because it does not include the costs of metabolism. We estimated the MI values from the functions fitted to the embryo dry mass-stage of development regressions by directly estimating the predicted mass of the egg when fertilized (stage 0) and the mass of the offspring at birth (stage 50).

We characterized the size distribution of mature males with PROC Univariate (SAS Institute, 1999). Our main interest was to determine whether or not the size distribution deviates from a normal distribution in a fashion that is consistent with a genetic polymorphism for age and size at maturity, as seen in other poeciliids (Kallman, 1989).

## RESULTS

### Female life history traits

The size distributions of reproducing females (Table 1) ranged from a minimum of 35.26 mm in collection 1 to a maximum of 60.49 mm in collection 3. We were unable to dissect females smaller than the minimum sizes that were gravid in collections 1 and 3, so we cannot estimate the minimum size of reproducing females for those two collections. For collection 2, we found that females in the range 39.58–58.31 mm were gravid. Six females in the size range 28–39.52 mm were not gravid, so it appears that females began to reproduce when in the range 39–40 mm. This minimum size clearly varies among collections because many females smaller than 39 mm in collection 1 were pregnant. The number of developing broods of young per female ranged from 1–6 across the three collections. We also observed that all gravid females had distinct genital papillae that were not present in nongravid females.

The average number of developing litters per female ranged from 2.9 in collection 3 to 4.9 in collection 1 (Table 1). The number of developing litters was positively correlated with female standard length in all three collections, significantly so in collections 1 and 2 [slopes ± SE (probability that slope exceeds 0): collection 1 = 1.82 ± 0.81 (0.0370); collection 2 = 2.95 ± 1.19 (0.0235); collection 3 = 1.53 ± 1.08 (0. 1741)]. We therefore included length as a covariate when comparing localities. A preliminary analysis showed that there were no Locality × female length interactions ($F_{2,52} = 0.22$, $P = 0.81$) for the regression of the number of developing litters on female length, so we assumed that the slopes of these regressions were equal in the subsequent analysis of covariance. The analysis of covariance revealed significant variation among collections ($F_{2,54} = 19.75$, $P < 0.0001$) and that the significance was attributable to a smaller number of developing litters in collection 3 relative to collections

**Table 1.** Descriptors of the life histories of females from collections 1–3

| Collection | Size range (mm) | Number of litters (mean ± SE) | Number of embryos per litter (mean ± SE) | Total number of embryos (mean ± SE) | Matrotrophy index (fixed effect) | Dry mass at stage 50 (mg) |
|---|---|---|---|---|---|---|
| 1 | 35.4–43.6 | 4.9 ± 0.25 | 4.7 ± 0.45 | 22.1 ± 1.8 | 4.03 (4.17) | 3.6 |
| 2 | 39.6–58.3 | 4.6 ± 0.18 | 3.6 ± 0.33 | 17.1 ± 1.3 | 3.38 (3.47) | 3.1 |
| 3 | 44.6–60.5 | 2.9 ± 0.23 | 4.9 ± 0.42 | 13.4 ± 1.7 | 4.18 (4.02) | 4.0 |

Size range (mm) is the range of sizes (standard length) of reproducing females. Number of litters, the least-square mean and standard error of the number of developing litters of young per female; number of embryos per litter, the least square means for average litter size; total number of embryos, the least square mean and standard error for the total number of developing young. All of the above least square means were derived from analyses of covariance that included length as a covariate. The matrotrophy index is the estimated value for the ratio of the estimated mass of embryos at stage 50 (advanced, near birth) divided by the estimated mass of an egg at stage 0 (mature egg ready to be fertilized). These values are derived from the regression of embryo dry mass on the stage of development in models that include individuals as a random effect. The value in parentheses are derived from fixed effect models that consider each litter as an independent observation. Mass at stage 50, or the projected mass of advanced embryos from these regressions, is an estimate of the dry mass of young at birth.

1 and 2 (Table 1) (Bonferroni adjustment for multiple comparisons among means: $P$ for locality 3 vs. 1 or 2 < 0.0001; $P$ for a comparison of 1 and 2 = 0.83).

The number of young per litter was not correlated with the stage of development of the young, so we evaluated litter size as either the mean number of young per litter in each female or as the total number of developing young per female. Mean litter size was positively correlated with female size in all three collections, significantly so in Locales 1 and 2 [slope ± SE (probability that slope exceeds 0): collection 1 = 2.40 ± 0.71 (0.0037); collection 2 = 3.33 ± 0.62 (< 0.0001); collection 3 = 0.63 ± 0.39 (0.1271)]. There was no significant heterogeneity among collections in the female length–mean litter size regressions ($F_{2,52} = 0.05$, $P = 0.9534$) so we compared collections with an analysis of covariance, with female length as a covariate, assuming homogenous slopes. There were significant differences among collections ($F_{2,54} = 5.93$, $P = 0.0047$) because the average, size-adjusted litter size was smaller in collection 2 than in collections 1 and 3. The difference between collections 1 and 2 was significant ($P = 0.0155$) based on a Bonferroni adjustment for multiple comparisons among means. None of the other pairwise comparisons were significant. This analysis was performed on log-transformed data, which normalized the residuals. The size-adjusted means in Table 1 were derived from the corresponding analysis on untransformed data.

The results for the total number of developing embryos carried by each female were similar, except that there tended to be a stronger and more consistent positive correlation between female size and embryo number [slopes ± SE (probability that slope exceeds 0): collection 1 = 0.56 ± 0.11 (< 0.0001); collection 2 = 0.53 ± 0.11 (< 0.0001); collection 3 = 0.23 ± 0.10 (0.0278)] (Fig. 1). There were also significant differences among localities in total fecundity but the rank-order was different from the analysis on the number of young per litter because the number of developing litters dominated in determining the total number of developing embryos. Locality 1 had the highest total fecundity followed by Locality 2 then Locality 3 (Table 1). The only significant difference in pairwise comparisons, with a Bonferroni adjustment for multiple comparisons, was between localities 1 and 3.

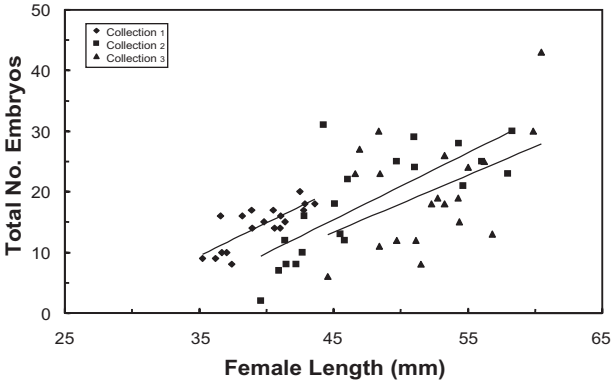


**Figure 1.** Female size–total fecundity regressions: the total number of developing embryos per female, which is the sum of the number of embryos in each litter that the female is carrying, is plotted against the female's standard length.

### MATERNAL PROVISIONING

The mass of embryos increased with the stage of development in all three collections (Fig. 2). The

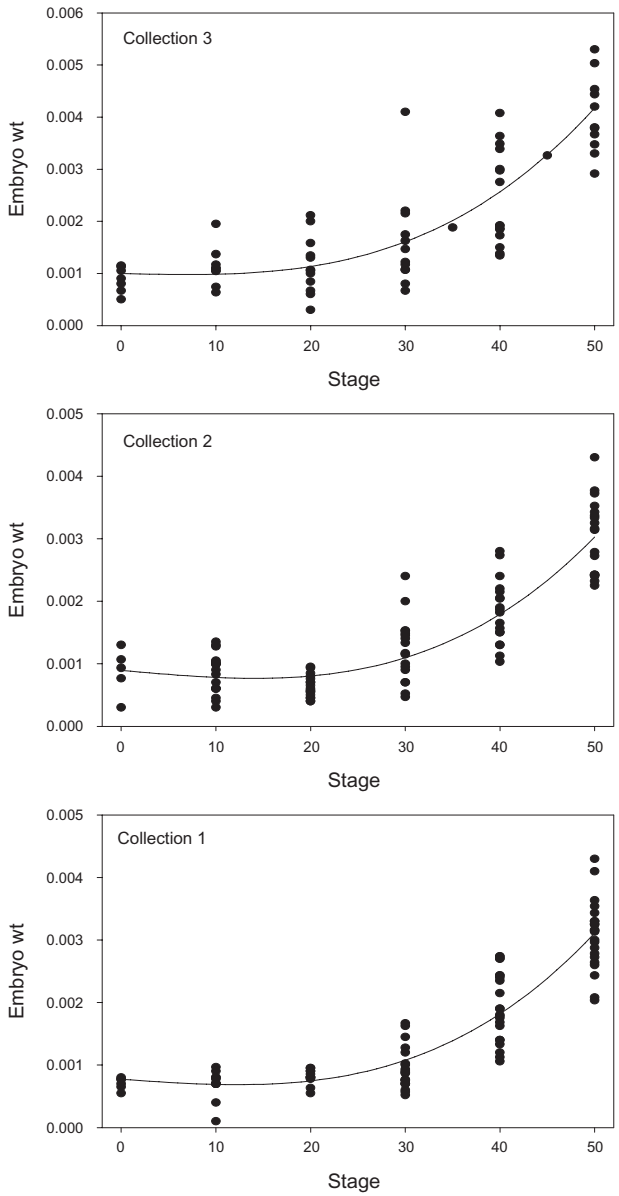


**Figure 2.** Stage of development–embryo mass regressions: the dry mass (mg) of developing embryos is plotted against their stage of development. Stage '0' corresponds to a mature egg that is ready to be fertilized. Stage 50 is an advanced embryo ready to be born. The curves are derived from a mixed model applied separately to each collection and that treats individuals as a random effect.

initial stepwise analysis gave the best model as $y = c_0 + c_1 \times \text{stage} + c_2 \times \text{collection} + c_3 \times \text{stage}^3 + c_4 \times \text{st}$ age) (Collection). Due to a failure to converge to a solution only the intercept $c_0$ could be entered as a random effect (i.e. individual females differ in the value of $c_0$): we shall refer to this as the 'full model' (Table 2). This model was superior to one that either excluded the collection or individuals (Table 3). Because of the significant heterogeneity among collections in the relationship between stage of development and embryo mass, we fitted a separate model to each collection (Table 4) and generated an estimate for MI from each collection (we include the estimates from the fixed effects model to show that, although there were significant differences among females within a collection for the pattern of mass change of embryos during development, this heterogeneity does not greatly affect the estimated value of MI). Our estimated values ranged from 3.38 for collection 2 to 4.18 for collection 3; these values suggest that embryos increased in dry mass three- to four-fold between fertilization and birth.

Hubbs (1950) described each developing embryo in his single mature female as being enclosed in an envelope. Likewise, we found that each embryo was contained within a relatively thick follicle that was superficially similar in appearance to the follicles seen in species of *Poeciliopsis* that have high matrotrophy indices, including *Poeciliopsis presidionis*, *Poeciliopsis turneri*, and the three species of *Aulophallus* (D. Reznick, pers. observ.; Turner, 1940a; Reznick, Mateos & Springer, 2002).

### Size distribution of mature males

Only collections 2 and 3 had sufficient numbers of mature males for us to analyse their size distribution ($N = 18$ for collection 2, $N = 30$ for collection 3). The common features of both samples are that the size distribution of mature males deviates significantly from a normal distribution and that both distributions are platykurtotic (Shapiro–Wilk test of normality: collection 2, $W = 0.884589$, $P = 0.0378$; collection 3, $W = 0.923641$, $P = 0.0334$) (kurtosis scores: collection 2 = –1.32; collection 3 = –1.08). The length–frequency histograms show that the size distribution is bimodal for the locality 2 data, with modes at approximately 33 and 45 mm (Fig. 3). The distribution for collection 3 is more uniform, with 'thicker tails' than would be the case for a normal distribution. The largest immature males in all collections were as big as or bigger than the mature males (data not shown), which is what is seen in other poeciliids, all of which are believed to have determinate or near-determinate growth in males.

## DISCUSSION

### Female life history

Our much larger data set shows that Hubbs' conclusion, based on a single female, accurately represents the mode of reproduction of the species. Nearly all of the females that we dissected contained multiple

**Table 2.** Analysis of variance of full, mixed model for the relationship between the dry mass of individual offspring and the stage of development

| Coefficient | d.f. | Sum of squares | Mean square | $F[P > (F)]$ |
|---|---|---|---|---|
| Stage | 1 | 0.000164 | 0.000164 | 598.0 (0.0001) |
| Collection | 2 | 0.000014 | 0.000007 | 26.1 (0.0001) |
| Stage | 1 | 0.000041 | 0.000041 | 148.6 (0.0001) |
| Stage × collection | 2 | 0.0000029 | 0.0000014 | 5.25 (0.0059) |
| Residual | 236 | | | |

The *r*-squared for the full model is 0.757.
d.f., degrees of freedom.

**Table 3.** Log-likelihood ratio tests of the full model with modelsthat exclude either locality or individual (= fixed effects model)

| Reduced model | d.f.* | Log-likelihood ratio | *P* |
|---|---|---|---|
| Collection omitted | 5 | 42.62 | < 0.001 |
| Fixed-effects | 8 | 31.32 | ≤ 0.001 |

*There are 10 degrees of freedom (d.f.) in the full model.

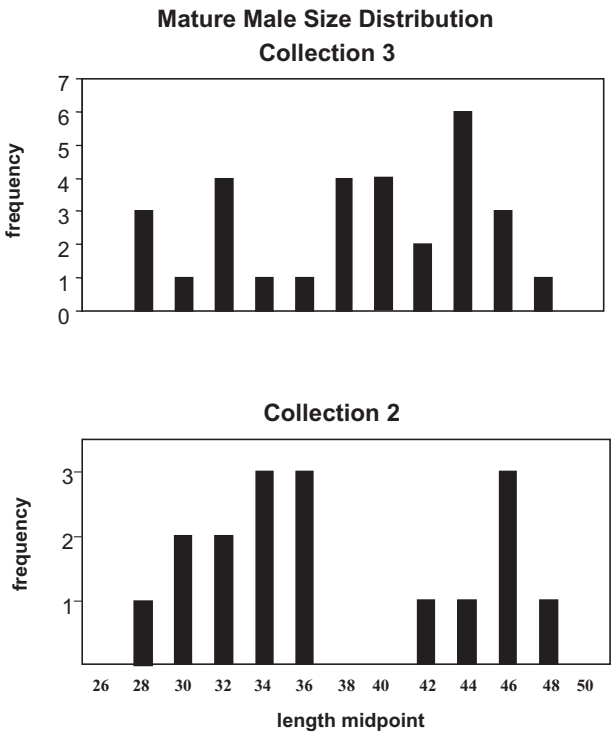


**Figure 3.** Size distribution of mature males: mature males, so defined by the complete metamorphosis of the anal fin into the gonopodium, are represented by their standard length.

broods of young in discretely different stages of development, indicating that the species has superfetation. The maximum number of six litters that we observed corresponds to his observation of six litters in his single observation. This level of superfetation is near the maximum observed for the family Poeciliidae (Reznick & Miles, 1989). We also established that this species has a moderate amount of postfertilization maternal provisioning since embryos increase in dry mass by a factor of three to four between fertilization and birth. This increase in dry mass is accompanied by a conspicuously thickened follicle that is superficially similar in appearance to the 'follicular pseudoplacenta' described for the genus *Poeciliopsis* by Turner (1940a).

Our statistical comparisons of the three collections show that there is often significant variation among populations in basic features of the life history, including the number of developing litters, litter size, and the total number of developing offspring. Because these descriptions are based on preserved material alone, we have no way of determining whether these differences represent genetic differentiation among populations/years or environmentally induced variation.

*Xenodexia* is similar to three of the four other species with superfetation and placentation that have been described thus far because it displays a strong correlation between female size, brood size, and the number of developing broods. Cheong *et al*. (1984) and Thibault & Schultz (1978) found such size-fecundity correlations for *Heterandria formosa*, *Poeciliopsis lucida*, and *Poeciliopsis prolifica*, respectively. There was no correlation between female size and the total number of developing young in wild-caught samples of *Poeciliopsis turneri* (Thibault & Schultz, 1978).

## Male size distribution

The non-normal size distribution, if accompanied by the indeterminate growth that is typical of the family, suggests that these fish may be similar to species in the genus *Xiphophorus* (Kallman, 1989) in harbouring a genetic polymorphism for age and size at maturity in

**Table 4.** Models fit to each locality by itself. These models include individuals as a random effect

| Collection | Intercept ± SE | *t* | Stage ± SE | t($P > \|t\|$) | Stage‡ | *t*($P > \|t\|$) |
|---|---|---|---|---|---|---|
| 1 | 0.0007 ± 0.0001 | 5.04* | –0.0089 ± 0.0079 | –1.12† | 22.48 (2.59) | 8.68[a] |
| 2 | 0.0009 ± 0.0001 | 6.50* | –0.0124 ± 0.0079 | –1.57† | 22.93 (2.68) | 8.36[a] |
| 3 | 0.0010 ± 0.0002 | 4.61* | –0.0023 ± 0.0128 | –0.18† | 25.00 (4.63) | 5.40[a] |

*$P < 0.0001$.
†Not significant ($P > 0.05$).
‡Numbers in parentheses are standard errors.

males. Either polymodal size distributions or genetic variation for age and size at maturity is also now known for guppies (*P. reticulata*) (Reznick *et al*., 1997), sailfin mollies (*Poecilia latipinna*) (Travis, 1994), *Phallichthys quadripunctata* (Kolluru & Reznick, 1996), and *Phalloceros caudimaculatus*) (Arias & Reznick, 2000). The phylogenetic distribution of the known occurrences of the genetic polymorphism and the suggestion, based on non-normal size distributions, that it occurs elsewhere in the family, implies that polymorphisms may be a widespread feature of the Poeciliidae. The work on *Phallichthys* quadripunctata (Kolluru & Reznick, 1996) demonstrates that such variation is not necessarily attributable to the same *p*-allele mechanism that has been described for *Xiphophorus* (Kallman, 1989); in that study, a bimodal size distribution was attributable to a mix of genetic and environmental effects. Such variation serves as an indicator that this is a phenomenon that is worthy of further investigation.

## Implications for life history evolution within the Poeciliidae

If *Tomeurus* is the sister taxon to the rest of the family (Rosen & Bailey, 1963; Parenti, 1981; Lucinda & Reis, 2005) and if the closest outgroups were egg-layers (Parenti, 1981), then the most likely pattern of life history evolution would be for the common ancestor of the family to have been an egg layer with internal fertilization, for livebearing to have evolved in the common ancestor of the remainder of the subfamily, and then for superfetation and matrotrophy to have evolved multiple times within the family (Reznick & Miles, 1989). If *Xenodexia* is nested within the family, as in the classification of Lucinda & Reis (2005), then it would represent an independent origin of superfetation and matrotrophy because the taxa that are sister to it (*Xiphophorus*, *Carlhubbsia*, *Quintana*) are lecithotrophic and lack superfetation (Reznick & Miles, 1989).

The DNA-based phylogeny for the family Poeciliidae of Hrbek *et al*. (2007) suggests alternative patterns of life history evolution. They found strong support for a basal split between *Xenodexia* and the remaining poeciliids using 4333 aligned base pairs of mitochondrial DNA from 14 loci and 1549 aligned base pairs of nuclear DNA from the RAG1 locus. Their analysis included 48 ingroups and seven outgroups with a range of diversity that is comparable to the morphological analyses of Lucinda & Reis (2005). One alternative interpretation is that the common ancestor of the family was an egg layer, like *Tomeurus*, and that there was an independent origin of superfetation and matrotrophy in the long-branch that leads to *Xenodexia*. A second alternative is that the common ancestor of the family was a livebearer with both superfetation and matrotrophy, like *Xenodexia*, then that superfetation and placentas were lost and repeatedly re-evolved within the family. This alternative also implies that egg-laying re-evolved in *T. gracilis*. Other alternatives are for the common ancestor to have had one or two of what had been considered the three derived life history traits in a tree that has *Tomeurus* as sister to the rest of the family. Whichever alternative is correct, the implication is that one or more traits that are considered to be derived based on a phylogeny with *Tomeurus* as the sister taxon to the rest of the family would instead have been ancestral then lost in some of the subsequent branches of the family tree. The plethodontid salamanders provide a precedent for a reversal from what had been thought to be a derived to a primitive mode of reproduction; in that case, it appears that there was at least one reversal from an ancestor with direct development from egg to a terrestrial juvenile to a descendent with an aquatic larval phase and metamorphosis to a terrestrial phase (Mueller *et al*., 2004).

We cannot discriminate among these alternatives because there are still incongruences between molecular and morphological analyses in positively identifying the basal split within the Poeciliidae. A complete reconstruction of the pattern of life history evolution within the family Poeciliidae also demands that we firmly establish its sister group as there are discrepancies here as well (Parenti, 1981; Meyer & Lydeard, 1993). Regardless of the nature of the common ancestor and the pattern of life history evolution within the

family, *Xenodexia* represents an independent occurrence of the combination of superfetation and placentation in the sense that the other species of Poeciliidae that have this same life history did not inherit it from a common ancestor to *Xenodexia*. This combination of life histories is also found in *Heterandria formosa* and in six species with three independent origins in the genus *Poeciliopsis* (Reznick *et al*., 2002; Reznick & Miles, 1989). This diversity highlights the potential of the family Poeciliidae for illuminating the evolution of complex traits.

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