

Equal fecundity in asexual and sexual mollies (*Poecilia*)

Ingo Schlupp · Angelika Taebel-Hellwig ·
Michael Tobler

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Abstract The evolution and maintenance of sexual reproduction is still one of the major unresolved problems in evolutionary biology. Sexual reproduction is fraught with a number of costs as compared to asexual reproduction. For example, sexuals have to produce males, which—given a 1:1 sex ratio—results in a two-fold advantage for asexuals that do not produce males. Consequently, asexuals will outperform and replace sexuals over time assuming everything else is equal. Nonetheless, a few cases of closely related asexuals and sexuals have been documented to coexist stably in natural systems. We investigated the presence of a two-fold cost in a unique system of three closely related fish species: the asexual Amazon Molly (*Poecilia formosa*), and two

sexual species, Sailfin Molly (*P. latipinna*) and Atlantic Molly (*P. mexicana*). Amazon Molly reproduce gynogenetically (by sperm dependent parthenogenesis) and always coexist with one of the sexual species, which serves as sperm donor. In the laboratory, we compared reproductive output between *P. formosa* and *P. mexicana* as well as *P. formosa* and *P. latipinna*. We found no differences in the fecundity in either comparison of a sexual and the asexual species. Under the assumption of a 1:1 sex ratio, the asexual Amazon Molly should consequently have a full two-fold advantage and be able to outcompete sexuals over time. Hence, the coexistence of the species pairs in nature presents a paradox still to be solved.

Keywords Poeciliid · Asexual · Recombination · Fecundity

I. Schlupp · A. Taebel-Hellwig
Universität Hamburg,
Zoologisches Institut und Zoologisches Museum,
Martin-Luther-King Platz 3,
20146 Hamburg, Germany

M. Tobler
Departments of Biology and Wildlife and Fisheries
Sciences, Texas A&M University,
2258 TAMU,
College Station, TX 77843, USA

Present Address:
I. Schlupp (✉)
Department of Zoology, University of Oklahoma,
713 Van Vleet Oval,
Norman, OK 73019, USA
e-mail: schlupp@ou.edu

Introduction

The evolution and maintenance of sexual reproduction is one of the major unsolved problems in evolutionary biology (Maynard Smith 1971; Williams 1975; Maynard Smith 1978; Bell 1982; West et al. 1999; Keightley and Otto 2006; Roze and Barton 2006). Sexual reproduction is thought to be costly for several reasons, and one of these costs is the production of males. While sexual species have to produce males, most asexual species produce only females. The advantage of the asexuals is two-fold if

the sexual species has a 1:1 sex ratio, and males only supply sperm, return nothing to the females, and provide no paternal care (Dawson 1995). All else being equal, asexual populations consequently have double the intrinsic growth rate of sexual populations, and an alternative reproductive strategy producing only females should invade and rapidly spread in sexual systems (Lively and Lloyd 1990).

Despite the difference in population growth rates, closely related asexual and sexual organisms have been shown to coexist in natural systems. Consequently, this begs the question whether asexuals can indeed capitalize on a two-fold advantage or whether some factors actually decrease their reproductive potential. Life history theory provides a framework for addressing these questions and allows us to compare the fitness of asexual and sexual species (Stearns 1992; Roff 2002). Such life history comparisons have been instrumental in determining if sexuals and asexuals produce an equal number of offspring in specific situations and have produced varying results in past studies. For example, sexual and asexual *Potamopyrgus* snails have an almost identical reproductive output (Jokela et al. 1997). Lamb and Willey (1979) reported lower hatching rates in parthenogenetic *Drosophila*. In reptiles, a study comparing sexual and parthenogenetic *Aspidocelis* lizards found no difference in fecundity between the two reproductive forms (Congdon et al. 1978), while asexual geckos (*Heteronotia*) have a 30% smaller size corrected fecundity as compared to several sexual congeners (Kearney and Shine 2005). Finally, in fishes, asexual and sexual topminnows of the genus *Poeciliopsis* have roughly similar fecundity when in mixed populations (Weeks 1995), but in monocultures the sexual *Poeciliopsis monacha* has a higher fecundity. The reproductive output of both sexuals and asexuals was strongly affected by density and was higher in lower densities.

In the present study we evaluated the “two-fold advantage” for an asexual species by comparing number of offspring in a well-described sexual/asexual mating system of mollies. *Poecilia formosa*, the Amazon Molly, is a gynogenetic species of livebearing fish (Hubbs and Hubbs 1932). It is a natural hybrid species between *P. mexicana* and *P. latipinna* (Hubbs and Hubbs 1932; Avise et al. 1991; Schartl et al. 1995b) that originated approximately 120 000 generations ago (Schartl et al. 1995b).

Amazon Mollies are clonal, all female, but require sperm to trigger embryogenesis (Schlupp et al. 1998; Schlupp 2005). Almost all Amazon Mollies are diploid, but occasionally males do make a genetic contribution, either in the form of a whole haploid sperm genome leading to triploids (Lampert et al. 2005) or as microchromosomes (Schartl et al. 1995a). These phenomena contribute to the genetic diversity within the species (Schlupp 2005). Males of the two parental species provide sperm in natural habitats as Amazon Mollies live in sympatry with *P. mexicana* in northeastern Mexico and with *P. latipinna* in southern and central Texas (Schlupp et al. 2002; Costa and Schlupp in press). This leads to a highly dynamic system, in which male choice plays an important role for the coexistence of host and sperm-parasite (Schlupp 2009). The stable coexistence of asexual and sexual mollies is not predicted by theory: if the asexual Amazon Mollies actually have the twofold advantage of not producing males, they should quickly outcompete their sexual hosts, just to subsequently disappear themselves due to the lack of sperm donors (Schlupp 2005).

In poeciliids, males make no contribution to reproduction other than sperm (Constanz 1989; Schlupp 2005a; Schlupp 2009). Also, sex ratios have been reported to be about equal at birth in sexual poeciliids (Snelson and Wetherington 1980). Consequently, comparing the fecundity of asexuals and sexuals serves as a valid first approximation of whether a two-fold advantage for asexuals is present in the system. We compared the fecundity between asexuals and sexuals in two different experiments highlighting different aspects. In the first experiment, we compared fecundity between females of *P. formosa* and *P. mexicana* born in the laboratory, and raised as virgins, while controlling for male effects. In the second experiment, we collected females of *P. formosa* and *P. latipinna* in the field to measure fecundity in females that matured in their natural environment and were inseminated by males in the field.

Material and methods

Laboratory reared females

For the first experiment, we used *P. mexicana* (IV/5) collected near Tampico (Tamaulipas, Mexico) and *P.*

formosa (D1) collected in Brownsville (Texas, USA). Both populations were collected in 1994 and since maintained in the laboratory. Stocks were maintained as large, randomly outbred populations at the University of Hamburg (Germany). All fish were fed *ad libitum* amounts of standard flake food supplemented with *Artemia* naupliae. They were kept under an artificial 12:12 h light cycle at 25° to 28°C.

For the experiment, we isolated virgin females of both species, all of which were at least 30 mm long, in 5 l aquaria. All aquaria were randomly placed in a rack in a large fish room. To start an individual trial, a sexually mature *P. mexicana* male from a stock tank was added to the tank. Offspring were counted immediately after they were detected (usually in the morning) and removed. After three broods, a female of the other species replaced the female, and brood sizes were recorded as for the first female. The order in which a male encountered the females was random. This paired design was used to minimize potential male effects and provide a direct, pairwise comparison of female reproductive output in the two species using the same individual male. Males and females were not replaced if they died during the experiment. If a male died after a female had two (or three) consecutive broods, the female was kept in the experiment until she had one additional brood. This was possible since females are able to store sperm (Constanz 1989). In all other cases, the trials were terminated as incomplete.

For each female, the average brood size was calculated. We also recorded female body size by measuring the standard length to the nearest millimetre. Females were approximately the same size, but we still corrected for size differences by dividing average clutch size by standard length (assuming a linear relationship between the two variables) and used the corrected variable in the analysis. Comparisons between the two species were carried out using a non-parametric Wilcoxon test, because we used a paired design by letting the same male provide sperm for both types of females.

Field collected females

For our second experiment, adult *P. latipinna* and *P. formosa* females were collected from a natural population (Lincoln Park, Brownsville, Texas, USA) in June 2005, where both species co-occur in the

same habitat. Fish were transported to a greenhouse at the Aquatic Research Facility of the University of Oklahoma in Norman (Oklahoma, USA). Females were housed individually in plastic tanks (4 l). A flow-through system using untreated well water was used to maintain proper water quality. Naturally growing algae and mosquito larvae served as food basis, which was supplemented with standard flake food three times a week. The experiment was started on 7 July 2005 and terminated on 25 November 2005. All females were checked for offspring daily in the morning hours. For each brood, we counted the number of offspring. At the end of the experiment, we measured female standard length to the closest millimetre. For data analysis, we compared size corrected mean brood sizes using a Mann-Whitney U-test.

Results

Laboratory reared females

The pairs of females used in our experiment did not differ significantly in median size [*P. formosa* SL: 37 mm (IQR: 9 mm); *P. mexicana* SL: 40 mm (IQR: 6 mm); Wilcoxon test: $N=9$, $Z=-0.775$, $P=0.438$; one size for a *P. formosa* was not recorded by accident]. Median male size was 29 mm (IQR: 9 mm). The size corrected median number of offspring was 0.352 mm^{-1} (IQR: 0.118 mm^{-1}) for *P. formosa* and 0.342 mm^{-1} (IQR: 0.166 mm^{-1}) for *P. mexicana* (Table 1, Fig. 1a). We compared the reproductive output of the females using a Wilcoxon-test and found no significant difference ($N=9$, $Z=-0.889$, $P=0.374$). To compare the number of female offspring between the two species, we divided the number of female offspring of *P. formosa* (0.342) by the number of female offspring of *P. mexicana* ($0.352/2=0.176$; assuming a 1:1 sex ratio) and found an average 1.94-fold advantage for the asexuals given the conditions of our experiment.

Field collected females

Females used in our experiment did not differ significantly in median size [*P. formosa* SL: 45.0 mm (IQR: 7 mm), $N=13$; *P. latipinna* SL: 44.5 mm (IQR: 5.5 mm), $N=26$; Mann-Whitney-U

Table 1 Descriptive statistics of fecundity in the three *Poecilia* species

	Mean±SD	Min	Max
Experiment 1			
<i>P. mexicana</i>	15.0±4.8	1	48
<i>P. formosa</i>	14.0±4.8	1	27
Experiment 2			
<i>P. latipinna</i>	25.2 ± 9.9	1	67
<i>P. formosa</i>	24.6 ± 11.8	1	55

test: $Z=-1.198$, $P=0.243$]. All females but two *P. formosa* and one *P. latipinna* had at least one brood during the experiment. The size corrected median number of offspring for *P. formosa* was 0.601 mm^{-1} (IQR: 0.317 mm^{-1} , $N=11$) and 0.573 mm^{-1} (IQR: 0.246 mm^{-1} , $N=25$) for *P. latipinna* (Mann-Whitney-U test: $N=36$, $Z=-0.206$, $P=0.839$; Fig. 1b; Table 1). In the test period, there was also no difference in the number of clutches [*P. latipinna*: median (IQR): 2 (1); *P. formosa*: 2 (2); Mann-Whitney-U test: $N=39$, $Z=-0.694$, $P=0.527$] or the total number of offspring produced [*P. latipinna*: median (IQR): 59.5 (53.75);

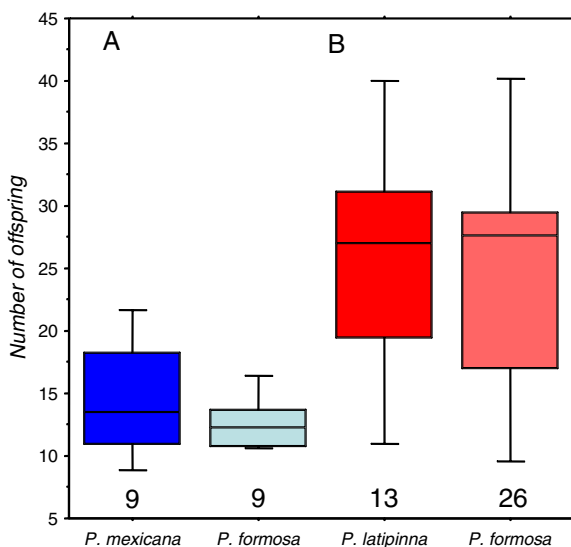


Fig. 1 Box plots of the mean numbers of offspring per female from two experiments. The numbers are not size corrected but there was no significant size difference among asexual and sexual females in either experiment, but the wild-caught *P. formosa* and *P. latipinna* were on average larger, which is reflected in brood size. **A** Study with laboratory raised fish comparing *P. mexicana* and *P. formosa*, and **B** study with field collected females comparing *P. latipinna* and *P. formosa*

P. formosa: 51 (51); Mann-Whitney-U test: $N=39$, $Z=-0.879$, $P=0.384$] between the two species. In this experiment, interbrood intervals were recorded for the subset of females that had more than one brood as 47 (6.5; median and IQR) for *P. latipinna* ($N=20$) and 43 (9.5) for *P. formosa* ($N=9$). The two species did not differ significantly (Mann-Whitney-U test: $N=29$, $Z=-1.628$, $P=0.100$). To compare the number of female offspring, we again divided the number of female offspring of *P. formosa* (0.601) by the number of female offspring of *P. latipinna* ($0.573/2=0.287$; assuming a 1:1 sex ratio). Given the conditions of our experiment, this represents an average 2.09-fold advantage for the asexuals.

Discussion

In both experiments, asexual Amazon Mollies had the same fecundity as the closely related sexual species. Under the assumption that sex ratios in the sexual species have a sex ratio at birth of 1:1, this results in an overall 1.9-fold advantage in experiment 1 and a 2.1-fold advantage in experiment 2 for the asexual mollies under the specific experimental conditions. Hence, at least in terms of their fecundity, Amazon Mollies exhibit the full two-fold advantage over sexuals as predicted by theory. It is important to note that we are looking at fecundity measured as live births, which may differ from estimates based on counting fertilized eggs and also makes no assumptions about juvenile survival to reproductive age.

It is possible that the absolute numbers of offspring in our two experiments differ from those expected for females in a more competitive environment in natural habitats, but the reproductive potential does not seem to differ between asexuals and sexuals. These findings are in agreement with studies by Hubbs (1964) comparing the offspring numbers of *P. formosa* and *P. latipinna* in dissections and Balsano et al. (1985) comparing the reproductive output of females of *P. mexicana* and *P. formosa*. Our findings are also in agreement with findings in some other systems (see introduction). Presently, we do not have a compelling explanation for the difference in fecundity between the two experiments. Several reasons could account for this, including differences between field- and laboratory-reared individuals and differences in the feeding regimes

between the two experiments. Clearly, offspring number is only one life history variable contributing to lifetime reproductive success. This highlights two important aspects: (1) stability in the Amazon Molly mating system cannot be explained by a reduction in offspring number. (2) Other life history traits (e.g., interbrood interval, age at first reproduction, size at first reproduction, reproductive allotment, survival rate, and growth rate) require careful further investigation. In the *P. latipinna*/*P. formosa* system in Texas, adult female size was slightly larger in Amazon Molly (41.3 ± 8.8 mm, mean \pm se, $n=949$) as compared to Sailfin Molly (37.2 ± 5.8 mm, $n=1016$) (data from Heubel 2004). Interbrood interval has previously been found to be the same in sympatric *P. formosa* and *P. latipinna* though (Hubbs et al. 2002), and our data is in agreement with this. Consequently, our present results are preliminary, but a more complete investigation to understand the life histories of asexual and sexual mollies is currently in progress.

In absence of clear life history differences, the stable coexistence of asexual and sexual mollies remains a genuine paradox. What factors reduce the Amazon Molly's two-fold advantage and mediate stable coexistence of the two reproductive forms? Neonate survival has been found to be lower in Amazon Molly than in Sailfin Molly under food stress (Tobler and Schlupp 2010), which may strongly influence population dynamics. In a separate study, under benign conditions no differences were found (Hubbs and Schlupp 2008). More experiments are needed in the Amazon Molly system along with tests of existing models incorporating clonal competition (Pound et al. 2002) and ecological limitations (Doncaster et al. 2000; Schley et al. 2004). In addition, field studies and common garden experiments are needed to fully illuminate the role of life history variation for the stability of this mating system.

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