

Mate Choice and the Amazon Molly: How Sexuality and Unisexuality Can Coexist

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Abstract

One of the most complex and important problems in modern evolutionary biology remains to explain the evolution and persistence of sexual reproduction given the costs of sexuality. One way to study the relative strengths and weaknesses of sexuality and asexuality is to study the dynamics of the coexistence of sexual and asexual organisms. An excellent model system for such an approach is the unisexual Amazon molly, *Poecilia formosa*. This livebearing fish is clonal, but requires sperm to trigger embryogenesis. Amazon mollies need to obtain sperm from males of closely related species. In an effort to understand the apparently paradoxical coexistence of the Amazon molly with its sexual hosts, many theories were tested, but most seem to have little relevance to understanding stability, whereas male mating behavior is potentially very important.

Key words: *gonogenesis, male choice, Poecilia formosa, Poecilia latipinna, Poecilia mexicana, sperm*

Introduction

The evolution of sex and recombination is still considered a major problem in evolutionary biology (Maynard Smith 1978; Bell 1982; West et al. 1999). Theory suggests that sexuality is difficult to evolve when competing with asexuality, largely due to the cost of producing males (2-fold cost of males; Maynard Smith 1978). The underlying ecological problem is easily understood. Suppose a sexual and an asexual form share the same, somehow limiting habitat. The sexual females produce both male and female offspring, whereas the asexual females produce only daughters, which again have only daughters and so on (Maynard Smith 1978). Essentially, in every generation the asexual females produce twice the number of daughters as compared with sexual females. Assuming equal fitness for asexual and sexual females, the asexual species will very likely replace the sexual species. Of course, the assumption of equal fitness is crucial. If asexuals have strongly reduced fitness, the probability of them successfully invading a sexual population is reduced. The question of the maintenance of sex and recombination despite the cost can be tackled elegantly using the framework and tools of genetics (Kondrashov 1993), but is a multifaceted problem that needs to be addressed from a variety of perspectives, including behavior and ecology. Once sexuality has evolved as a reproductive strategy, it is equally puzzling that asexuality, if it somehow arises, does not successfully invade

and take over sexual populations due to faster population growth (see below). This question about the maintenance of sex has many facets, one of them the ecological and behavioral dynamics of the 2 different reproductive strategies. In the present review, I focus on the organismal, ecological side of the problem, highlighting recent work especially in behavior that can help us understand the precarious coexistence of asexual and sexual reproduction.

Notably, the advantage of sexual reproduction is only pertinent in the short run; very little disagreement exists about the long-term consequences of asexual reproduction, namely, decay of the genome and extinction (Muller 1964; Loewe and Lamatsch 2008). Extinction is of course also the fate of most sexual organisms. The extreme advantage of asexual reproduction at least in the short run begs the question of why in reality asexuality and clonality are exceedingly rare in metazoans (Suomalainen et al. 1987; Vrijenhoek et al. 1989). Two major ideas can potentially explain this pattern: either sex and recombination have a major short-term advantage, which remains to be identified, or the origin of asexual forms and their successful establishment among sexual organisms are exceedingly rare (Schlupp 2005).

Asexual organisms in general are a relatively small minority of all organisms (Suomalainen et al. 1987; Avise 2008). A subset of these organisms is characterized not only by asexuality but also by sperm dependence (Vrijenhoek

1994; Schlupp 2005; Schlupp and Riesch, forthcoming). In these cases, some disadvantages of asexuality (e.g., the accumulation of deleterious mutations) and some disadvantages of sexuality (e.g., having to mate) are combined in one organism. Sperm-dependent parthenogenesis is rare but extremely interesting to study because it allows us to view sexual and asexual reproduction as alternative strategies realized in typically closely related species in very similar (sometimes identical) habitats. In sperm-dependent species, the (often diploid) eggs cannot develop without a stimulus, and this stimulus is sperm. Sperm dependence can occur in 2 different versions: 1) gynogenesis, in which eggs cannot develop without a sperm stimulus, and 2) hybridogenesis, in which the sperm fuses with the egg (Vrijenhoek 1994). For the latter, the male chromosomes are removed from the egg during oogenesis via complex but little understood mechanisms (Tunner 1981). This leads to a hemiclinal mode of inheritance, with only the female half of the genome being inherited clonally. In this particular case, male genes are involved in expressing the phenotype but are not being passed on.

In gynogenesis, as found in the Amazon molly (*Poecilia formosa*), the focus of this review, typically sperm does not play any role beyond triggering embryogenesis and male genes are not incorporated, but in some cases, introgression of male genetic material has been reported. Introgression appears to happen when the cellular mechanisms destroying male chromosomes in the egg breakdown. Sometimes a whole male genome is added, leading to triploid lineages (Balsano et al. 1972; Rasch and Balsano 1989; Lamatsch et al. 2000; Lampert et al. 2005). In *P. formosa*, such triploids also reproduce via gynogenesis. In another sexual/asexual complex of fishes, *Poeciliopsis*, diploids are hybridogenetic, but triploids are gynogenetic (Vrijenhoek 1994). In *P. formosa*, the highest ploidy level reported was found in one tetraploid individual (Lampert et al. 2008). Another avenue leading to introgression is the partial incorporation of male chromosomes in the form of microchromosomes (Schartl et al. 1995). These are very small chromosomes that are found both in the laboratory and in nature (Lamatsch et al. 2004), and they are stably inherited (Nanda et al. 2007) and expressed (Schartl et al. 1997; Schlupp et al. 1998; Lamatsch et al. 2000). The evolutionary effects of this introgression are not well understood, but may make important contributions to the genetic diversity within *P. formosa*.

The Amazon molly—named after the all-female tribe from Greek mythology—is a natural hybrid species that produces only females and consequently is unisexual (Hubbs CL and Hubbs LC 1932). The maternal ancestor was the Atlantic molly, *Poecilia mexicana* (Avise et al. 1991), and the paternal ancestor was the Sailfin molly, *Poecilia latipinna* (Schartl et al. 1995). The (likely) single hybridization event happened approximately 280 000 years ago in the general area of Tampico, Mexico (Schartl et al. 1995; Möller 1999; Stöck M, Lampert KP, Möller D, Schlupp I and Schartl M. submitted). Although the actual evolutionary sequence is not known in this case, it should be noted that Amazon mollies represent a case of instantaneous hybrid speciation (Seehausen 2004; Mallet 2007). Curiously, so far it

has been impossible to create *P. formosa*-like, gynogenetic hybrids in the laboratory; crosses have so far always resulted in sexual F₁ offspring, although many of the F₁ females possess a trait that might make the evolution of gynogenesis more likely, namely diploid eggs (Lampert et al. 2007).

Amazon mollies are sperm dependent, which forces them into a parasitic relationship with a species that will provide sperm for successful reproduction (Hubbs CL and Hubbs LC 1932; Hubbs 1964; Schlupp 2005). Because Amazon mollies have internal fertilization, males need to copulate with females for insemination. Males from 2 species (*P. latipinna* and *P. mexicana*; Schlupp et al. 2002) are the main source of sperm for Amazon mollies. These host species are also the 2 species involved in the hybridization. A third species, the Tamesi molly, *Poecilia latipunctata* is also known to be a sperm donor (Niemeitz et al. 2002; Ptacek et al. 2005). This species, however, seems to play only a minor role as host (Tobler and Schlupp 2009). Amazon mollies occur in southeast Texas and northwest Mexico (Schlupp et al. 2002; Costa and Schlupp, forthcoming). The natural northern limit is the Nueces River, but recently Amazon mollies seem to be expanding their range northward (Martin D et al., personal communication). The southern limit is the Rio Tuxpan, Mexico. Both parental species have ranges that far exceed the area of sympatry with Amazon mollies, so that in both *P. latipinna* and *P. mexicana*, studies are possible comparing allopatric (not living with *P. formosa*) and syntopic (living with *P. formosa*) populations (Gabor and Ryan 2001).

Genetically, Amazon mollies are apomictic parthenogens and produce diploid eggs without meiosis (Rasch et al. 1982). Consequently, males mating with such females make an investment into mating without leaving behind offspring to which they are genetically related. The cost for such interactions is about the same for interactions with unisexual and sexual females: males of *P. latipinna* housed with either conspecific or Amazon molly females lost condition (a combined measure of weight and length) equally, whereas males from a control group kept with *Gambusia* females (another livebearing fish, but only distantly related, that is sexually ignored by *P. latipinna* males) gained condition (Schlupp I, Plante S, Chapman G, unpublished data). Hence, selection should act against males that engage in matings with Amazon mollies, if their conspecific competitors leave behind more offspring with their conspecific sexual females (Schlupp et al. 1994).

Chasing the Short-Term Advantage of Sex

As mentioned above, extant asexual hybrids may be rare not only because they may go extinct quickly but also because they are not formed very often. This could be the case because the genomic and ecological conditions for the successful hybridization of 2 species are very rare (Kearney et al., 2006; Stöck et al., submitted). Furthermore, the resulting unisexual species may be at risk of extinction at least early in the evolution of the new lineage due to chance events. Once unisexuals are formed though, they can be

ecologically very successful (Suomalainen et al. 1987; Vrijenhoek et al. 1989; Johnson et al. 1995). Rare formation, however, has no bearing on the stable coexistence of unisexual and sexuals as we find it in the Amazon molly complex. To explain this, either the sexual strategy has to have a major short-term advantage or the unisexual strategy has to have a major short-term disadvantage (or a combination of both) to bridge the 2-fold difference in fitness between unisexuals and sexuals.

Many ideas have been proposed that can account for this difference (Bell 1982; Kondrashov 1993), but no single one has sufficient scientific support to explain the evolution of sex or the coexistence of sexual and closely related asexual forms (West et al. 1999).

One approach to tackle this problem is to try to document differences in life-history traits. This could be important because reduced life-time reproductive output (e.g., via smaller brood sizes or longer interbrood intervals) might reduce the 2-fold advantage of asexuality. Preliminary studies (Schlupp I et al., Forthcoming and Riesch R, Tobler M, Plath M, Schlupp I, unpublished data) indicate that fecundity is equal for unisexuals and sexuals (both *P. mexicana* and *P. latipinna*). In addition, survival of neonates is not different under benign laboratory conditions (Hubbs and Schlupp 2008), but under food stress, survival of Amazon molly fry is reduced (Tobler and Schlupp, forthcoming). The latter might constitute an important disadvantage, but more data are needed here. The overall investment of mothers into their offspring (measured as reproductive allocation: percent body weight consisting of embryos) is also not different (Riesch R, Tobler M, Plath M, Schlupp I, unpublished data). Although many more studies, including carefully planned common garden experiments, need to be conducted, there is no clear disadvantage for *P. formosa* detectable in the current data.

Another very promising candidate is the “Red Queen” hypothesis (Van Valen 1973), which predicts an immediate advantage of being sexual, essentially because the highly diverse offspring resulting from sexual reproduction provide a moving target for parasites, whereas the genetically very uniform offspring of clonal females should be easily exploited by parasites. This hypothesis has strong empirical support from studies in several systems, for example in comparing triploid asexual snails with diploid sexual snails (Lively et al. 2004), but no support in the Amazon molly system. Here, an intensive field study comparing many different parasites revealed that sexual fishes (*P. latipinna*) have as many or even more parasites than syntopic asexual Amazon mollies (Tobler and Schlupp 2005; Tobler et al. 2005), although the Amazon molly has a reduced number of MHC class I and class IIB alleles present in the population (Schaschl et al. 2008). Consequently, the support for a “Red Queen” mechanism at work in this particular system is very weak.

In addition, recently, the generality of the “Red Queen” as explanation for the advantage of sexuality over asexuality has been called into question (Salathe et al. 2008; Tobler and Schlupp 2008). Clearly, in some systems, the support for a Red Queen mechanism is very strong (Lively et al. 2004)

and theoretical support is also strong, but in many comparative studies of sex and asex, other factors may account for or contribute to the reported differences in parasite load. Such factors may include differences in behavior, niche use, and immunology. Clearly, this is a contentious area, and more data are needed for a full evaluation of this mechanism.

Interestingly, some parasite infections like the black spot disease (caused by the metacercariae of trematodes) can influence female preferences in such a way that unisexual females, but not sexual females (*P. latipinna*) avoid males with black spots (Tobler et al. 2006). The adaptive value (if there is one) of this is not clear yet, but if males infected by black spot disease also carry other parasites, Amazon mollies (that may also have a less well-developed immune system) might avoid exposure by rejecting such males.

Obviously, abiotic factors can also contribute to stability if they selectively and negatively affect Amazon mollies. A recent study found that *P. latipinna* are less affected by low temperatures than Amazon mollies (Fischer and Schlupp 2009): *P. formosa* lose motion control at a higher temperature, making them more vulnerable to predators and mortality. If this translates into differential survival, this may provide a considerable advantage for *P. latipinna* in South Texas because of the winter temperatures there. In nature, Fischer and Schlupp (2009) found several instances of temperatures that could negatively affect Amazon mollies, but not Sailfin mollies. This hypothesis is congruent with a previous observation by Heubel et al. (2008), who found an effect of winter on population composition in the same area. Frequencies of Amazon mollies declined over the winter, after growing strongly over the summer. It is tempting to hypothesize that this effect is caused by temperature-mediated winter mortality.

It seems none of the effects described above provides an obvious explanation for the apparent stability of coexistence of Amazon mollies and their sexual hosts. Even combined they seem too weak, which leads back to male behavior as a potential contributor to stability.

The Role of Male Behavior: “Love Slaves of the Amazons” (Title of a 1957 Movie by Curt Siodmak)?

Males have the potential to drive sperm-dependent systems, like the sexual/asexual complex of *Poecilia*, because they could exercise male mate choice and thereby limit the population growth of the unisexual Amazon mollies. This is an unusual role for male choice, which is generally considered to be less important in sexual selection than female choice. Recent years, however, have witnessed an increased interest in male mate choice beyond the unusual situation of the Amazon mollies (Schlupp et al., 1991; Saether et al. 2001; Schlupp and Plath 2005; Simcox et al. 2005; Servedio 2007). A first theoretical model for the role of male mate choice was provided by McKay (1971) for the *Poeciliopsis* complex highlighting the potential role of

negative frequency dependent male mate choice. Males would strongly prefer sexual females as they become rare in the population, thereby limiting the mating success of the asexual females. Recent theoretical models have again highlighted the role of male choice for stable coexistence and illustrated the importance of male choosiness (Kokko et al. 2008; Heubel et al. 2009).

For males to have an influence on population dynamics, they have to be able to discriminate between a sexual and a unisexual female. This form of species recognition has been studied in detail and males are clearly capable of distinguishing between unisexual and sexual females (Schlupp 2009; reviewed in Schlupp et al. 1991). Hence, males indeed have a preference for conspecific females (Schlupp, 2005, 2009), but this preference can break down under certain circumstances: for example, if an unisexual female is sexually receptive (Parzefall 1973) and males can detect this chemically, the preference changes and males of *P. mexicana* will actually prefer receptive heterospecific females over nonreceptive conspecifics (reviewed in Schlupp 2009). Such observations are relevant in several ways. They indicate how dynamic mate choice can be and provide insights into the coevolutionary arms race (Dawkins and Krebs 1979) that might play out here: as males evolve to avoid costly matings with Amazon mollies, those evolve to obtain sperm despite male avoidance. Most importantly, the life-dinner principle (Dawkins and Krebs 1979) might apply here: although mating with a heterospecific female may be costly for a given male, not obtaining sperm is fatal for the Amazon molly. It should be noted, though, that Amazon mollies might be very slow to evolve, which may slow down the arms race or prevent it from occurring at all (Dries 2003).

Traditionally, male choice is studied by investigating mate preferences. These preferences are often studied as association times. To do this, the choosing individual is given the opportunity to be in spatial proximity with one of 2 alternative stimuli. If these stimuli are present at the same time, it is a simultaneous or binary choice test. The choosing subject can exercise “nonchoice” by not associating with any of the 2 stimuli and instead spend time in a central, neutral zone. Generally, this is an excellent approach as it integrates all kinds of sexual preferences (Jennions and Petrie 1997; Schlüter et al. 1998), but one has to consider that other, nonsexual preferences may also play a role (Gabor 1999). In practice, however, this distinction is probably not very meaningful, as a female approaching a male will elicit a mating response from that male. Alternatively, one can investigate male preferences at the level of sperm transfer and measure how much males invest into mating relative to the quality of the female. Essentially, by withholding sperm from Amazon mollies, males can drive this system. Studies of sperm priming, that measured how many sperm are being readied for insemination, for example revealed that *P. latipinna* males prime more sperm when visually stimulated by a conspecific, *P. latipinna* female, than when stimulated by an Amazon molly (Aspbury and Gabor 2004a, 2004b). This was echoed by another laboratory study in which males of *P. mexicana* were found to mate more often with and transfer more sperm to conspecific females (Schlupp and Plath

2005). Finally, in a field study, Riesch et al. (2008) found that Amazon mollies in Texas may be sperm limited because fewer of them had recoverable sperm in their genital tract as compared with Sailfin mollies (Riesch et al. 2008). This trend has recently been confirmed by a laboratory study on the same populations (Riesch R, Makowicz A, unpublished data). Of course, fewer sperm for the Amazon mollies—although probably reflecting male preferences—do not have to translate into reduced fitness per se, as typically far more sperm is inseminated than needed for fertilization (Hinze C, Schlupp I, in preparation). Nonetheless, all these examples clearly illustrate an important role of male mate choice in this system.

Complexity of Behavior

Sexual interaction, just like almost all other interactions happen in a social context. For most species, a situation allowing private communication only involving 2 or 3 individuals is unrealistic (McGregor and Peake 2000). Mollies form social groups, or shoals, probably trying to find safety in numbers. In addition, Amazon mollies form mixed groups with their sexual host species (Schlupp and Ryan 1996), meaning that what ultimately counts is that many matings happen for Amazon mollies in these groups. Nonetheless, many behavioral studies use interactions involving only 2 or 3 individuals as the prevalent paradigm, purposefully ignoring and removing interactions to have optimal control of the experimental conditions. This is a very powerful approach, but it limits our understanding of the importance of social interactions. Clearly, the social context is very important for the outcome of mating interactions. Recent years have seen major advances in this field, both in theoretical (McGregor et al. 1999; McGregor and Peake 2000; Matos and Schlupp 2004) and in experimental studies (McGregor and Peake 2000; McGregor et al. 2001; Matos and McGregor 2002). Social interactions may even include sexual harassment, but more specifically audience effects can occur in many different ways: female and male mate choice may be influenced by mate copying (see below), or eavesdropping may provide the bystander with a benefit by gleaning information from interactions of competitors (Matos and Schlupp 2004). Many more behaviors, like the time spent with potential mating partners or feeding behavior, are known to change as soon as some other individual is watching. Such audience effects have been documented many times in livebearing fishes, including mollies (Plath, Blum, Schlupp, et al. 2008; Plath, Blum, et al. 2008; Padur et al. 2009), other fishes (Oliveira et al. 2001; Dzieweczynski et al. 2005), and countless other taxa (Striedter et al. 2003). The bottom line of these advances is that social context matters (Figure 1). We should use these more complex models of mating interactions to better understand the circumstances and mechanisms that lead to heterospecific matings.

One particular way how the influence of social interactions can manifest itself is in so-called “nonindependent choice” (mate copying) (Westneat et al. 2000).

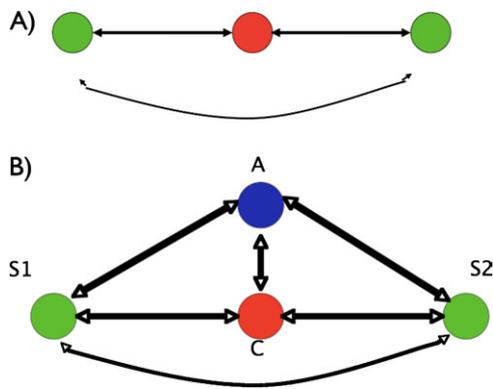


Figure 1. The role of context in animal behavior. (A) Possible interactions in a binary choice test (arrows) and (B) possible interactions with one audience individual added. A = audience, S = stimulus, and C = chooser.

In mate copying, a choosing individual is influenced in its decision by observing another individual choosing. In fishes, this phenomenon was first studied in guppies (*Poecilia reticulata*) (Dugatkin 1992). Later, this concept was applied to Amazon mollies to investigate if mate copying might provide an indirect benefit to males and thus lead to interactions with Amazon mollies. In support of this hypothesis, it turned out that Sailfin molly males were much more attractive to their conspecific females after they had been observed interacting with Amazon mollies (Schlupp et al. 1994; Heubel et al. 2008). This should make it easier for Amazon mollies to obtain the matings they need, potentially making the problem of stability worse.

Another important social influence on mate choice is sexual harassment and sexual conflict in general. In many animals, males try to force females to mate, resulting in sexual conflict (Magurran and Benoni 1994; Magurran and Seghers 1994). In livebearing fishes, sexual harassment and coercive mating are very common (Plath et al. 2007) and often multiple males pursue a female and try to force her to mate. This can have profound effects on female time budgets and on female fitness (Schlupp et al. 2001). Potentially sexual harassment can influence female opportunities for decision making. For example, if males face higher costs mating with conspecific females, they might switch over to less-resistant Amazon mollies.

Another facet was addressed in a recent study finding that male *P. mexicana* seem to attempt to deceive audience males by providing misleading information (Plath, Richter, et al. 2008). In an experiment, *P. mexicana* were given a choice between a conspecific and an Amazon molly female. In a first choice test without an audience male present, the males directed their first sexual behavior at the conspecific female. When they were retested in the presence of an audience male, however, they directed their first sexual behavior at the other, initially not preferred Amazon female. It seems as if males are trying to deceive their competitors and lead them into interactions with the “undesirable”

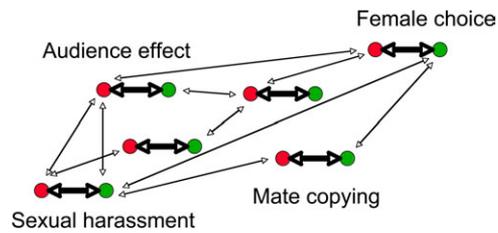


Figure 2. Social interaction network model. This model illustrates how multiple individual interactions that happen simultaneously can form a network of interrelated behaviors.

female (Plath, Richter, et al. 2008). Because earlier work had demonstrated that males of Sailfin mollies copy the mating decision of other males (Schlupp and Ryan 1997), this deception could actually lead to heterospecific matings.

In the sexual/unisexual mating system of the Amazon molly, there seems to be no simple answer to the question of how this complex remains stable. Although this is not surprising, it is perhaps unexpected that behavior, namely, male mate choice is currently the one candidate that has received the most empirical and theoretical support as a central regulatory mechanism in this system. In addition, it is intriguing that our understanding has made significant leaps by asking questions in the context of behavioral networks. It becomes increasingly clear that we are missing important aspects if we only focus on simple behavior in a 2-individual context without including the next level, behavior in its social context as a social interaction network (Figure 2) (Matos and Schlupp 2004). It might be beneficial to consider the explanatory power of this approach in more sexual/unisexual mating systems.

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