



Journal of Fish Biology (2011) **79**, 194–204

doi:10.1111/j.1095-8649.2011.03009.x, available online at wileyonlinelibrary.com

The impact of social context on male mate preference in a unisexual–bisexual mating complex

L. ALBERICI DA BARBIANO*[†], A. S. ASPBURY[‡], C. C. NICE[‡]
AND C. R. GABOR[‡]

**Department of Biology, Texas State University – San Marcos, TX 78666, U.S.A. and*

‡Department of Biology, Population and Conservation Biology Program, Texas State University – San Marcos, TX 78666, U.S.A.

(Received 11 December 2010, Accepted 14 April 2011)

Male sailfin mollies *Poecilia latipinna* were tested in five different treatments that varied in the relative frequency of heterospecific gynogens (Amazon molly *Poecilia formosa*) to conspecific females to determine whether social interactions among males within a population causes some males to mate with heterospecific females. Male *P. latipinna* inseminated a significantly higher proportion of conspecific females and fertilized a significantly higher number of conspecific eggs regardless of the treatment. Nonetheless, preference for conspecific females was not exclusive as a range of 20 to 50% of heterospecific females were fertilized. Social interactions among males may best explain the results and may therefore play an important role in the maintenance of unisexual–bisexual mating complexes.

© 2011 The Authors

Journal of Fish Biology © 2011 The Fisheries Society of the British Isles

Key words: frequency-dependent mating; gynogenesis; *Poecilia formosa*; *Poecilia latipinna*.

INTRODUCTION

Selection often favours individuals that evolve a mate preference for conspecific mates (Coyne & Orr, 2004). Mating with heterospecifics can result in a lowering of an individual's fitness because hybrid offspring may be non-viable, sterile or, in the particular instance of a unisexual–bisexual mating complex where the unisexual species reproduces by gynogenesis, the offspring will not inherit the paternal genes.

Unisexual–bisexual mating systems are found when an all-female species depends on the sperm of a closely related species for reproduction (Dawley & Bogart, 1989). Gynogenesis is a form of asexual reproduction used by several unisexual species that requires sperm from heterospecific males to trigger embryogenesis but no syngamy occurs and no genetic information of the male is inherited by the offspring (although some exceptions are known: Graf & Polls Pelaz, 1989; Schartl *et al.*, 1995a). Gynogenetic females are thus clonal and must live in sympatry with the sperm donor species (sexually reproducing host species).

[†]Author to whom correspondence should be addressed. Tel.: +1 512 245 3387; email: Laura_Alberici@txstate.edu

Here, the focus is on the Amazon molly *Poecilia formosa* (Girard 1859) and sailfin molly *Poecilia latipinna* (LeSueur 1821) unisexual–bisexual mating system to examine the effect of male–male interactions on the frequency of heterospecific mating. *Poecilia formosa* is an internally fertilizing ovoviviparous fish that lacks parental care. *Poecilia formosa* arose from a hybridization event between an Atlantic molly *Poecilia mexicana* Steindachner 1863 and *P. latipinna* c. 100 000 years ago (Avisé *et al.*, 1991; Schartl *et al.*, 1995b); although there is some controversy about this time period (Dries, 2003). The gynogenetic *P. formosa* must mate with males of its parental species to trigger embryogenesis, however, males of the parental species exhibit a stronger preference for conspecific females over heterospecific mates (Ryan *et al.*, 1996; Gabor & Ryan, 2001). Given the lack of benefits from mating with gynogenetic females (see Schlupp *et al.*, 1994 for exceptions), mating mistakes on the part of males in this and other unisexual–bisexual complexes are fundamental in maintaining the unisexual sperm-dependent species over time. Nonetheless, sometimes it may be advantageous for males to mate indiscriminately with both conspecific and heterospecific females depending on the cost of maintaining a strong mate preference (Heubel *et al.*, 2009).

Ecological or behavioural mechanisms must be in place to prevent unisexual species from outcompeting their sexual hosts given the two-fold reproductive advantage asexual species have over sexually reproducing species (Maynard Smith, 1968). Recently, Schlupp *et al.* (2010) found that *P. formosa* has equal fecundity to its host *P. latipinna* and suggested that ecological factors, such as higher mortality among juvenile *P. formosa*, prevent *P. formosa* populations from growing exponentially as predicted by theory (Schlupp *et al.*, 2010). An alternative, non-mutually exclusive hypothesis is that frequency-dependent male mate choice can cause fluctuations in the unisexual population, thus preventing the unisexual species from outcompeting their sexual hosts over time (Moore & McKay, 1971).

There is little knowledge about the effect of the composition of potential mating partners (*e.g.* the frequency of conspecific and heterospecific females in a shoal) on the plasticity of mate choice (Alonzo & Sinervo, 2001). Males may express frequency-dependent mate choice depending on social interactions with other males. For example, competition for conspecific mates may become more pronounced when conspecific females are less frequent in a population, therefore subordinate males may mate more with heterospecific females, because they have limited or no access to conspecific mates. In the unisexual–bisexual complex of *Poeciliopsis monacha-lucida* Miller 1960, Moore & McKay (1971) found that when only one male or multiple males of the same age, not organized in strict hierarchical scale, were given access to both conspecific and heterospecific females, only the eggs of conspecific females were fertilized. When males were of different ages (and therefore organized in a strict hierarchical scale), some heterospecific females were fertilized as well as conspecific ones (Moore & McKay, 1971). Kawecki (1988) showed that males that are subordinate tend to mate with heterospecific females because they have a shorter assessment time available, given that part of their mate searching time is limited by constant aggressive interactions with dominant males. When assessment time is limited, indiscriminate mate choice is more probable (Schmeller *et al.*, 2005).

Mate choice involves two processes: species recognition and mate quality recognition (Ryan & Rand, 1993). The two processes may interfere with each other when heterospecific females resemble high-quality conspecific females (Pfennig, 1998). In

the *P. formosa* and *P. latipinna* mating system, the hybrid *P. formosa* closely resembles females of their parent species. If males are not given enough time to assess the potential mates, then they may incur a mate quality-species recognition conflict. Indeed, male *P. latipinna* from some sympatric populations show a conflict between species and mate quality recognition when heterospecific females are larger and thus resembled high-quality conspecifics (Gumm & Gabor, 2005). In this system, large female size is preferred by males (Ptacek & Travis, 1997; Gabor, 1999), and larger females are more fecund (Farr, 1989).

In the *P. formosa* and *P. latipinna* system, male *P. latipinna* do not organize themselves in complex social dominance hierarchies, but large males have been observed to be dominant over smaller males (Baird, 1968; Woodhead & Armstrong, 1985; Travis & Woodward, 1989) and both sperm-dependent and host-species females prefer to mate and associate with larger males (Marler & Ryan, 1997; Ptacek & Travis, 1997; Gabor, 1999). Additionally, smaller males tend to resort to sneak copulations when large males are present and are chased away by larger males, who tend to show courtship behaviours towards females (Travis & Woodward, 1989). These aggressive interactions cause smaller males to have a shorter assessment time, which may result in smaller males mating with smaller females or even heterospecifics.

Most mate-choice studies performed on mollies have presented a male with a choice of two females and were not aimed at analysing the interactions among males (apart from Plath *et al.*, 2008). In the present study, whether social context influences male mate choice was tested. This question was approached by manipulating the relative frequency of conspecific and heterospecific females in mixed shoals of *P. formosa* and *P. latipinna* to assess whether the relative availability of conspecific females affected male–male interactions, potentially resulting in some males mismating more often and a higher proportion of heterospecific females being inseminated. The proportion of fertilized females of each species within each replicate was analysed as the response variable. If females of the unisexual species are very frequent in a population while conspecific females are rare, male–male competition for high-quality mates (*i.e.* conspecifics) will be high and larger host-species males should be more likely to secure those matings while smaller males may be more prone to mate with heterospecific females.

MATERIALS AND METHODS

Fishes were collected during the summer 2006 and 2008 from a locality in northern Mexico (25° 18' 0" N; 97° 51' 36" W) where *P. formosa* and *P. latipinna* occur in sympatry. Fishes were transported to San Marcos, TX, U.S.A. and housed in 2100 l outdoor tanks at the Biology Department greenhouse. Experiments were conducted during September to October 2006, March to October 2007, March to April 2008 and April 2010. Fish smaller than 20 mm standard length (L_S) were brought into the laboratory and reared indoors and kept on a 14L:10D cycle. Males were removed to make sure that all tested females were virgins. Poeciliid males develop a gonopodium *via* fusion of the anal fin rays when they become sexually mature, making it easy to identify and remove males from the rearing tanks before they are fully matured. Poeciliid females can store sperm up to several months (Constantz, 1989); therefore, virgin females were used to ensure that any inseminations took place during the trials. Fishes were fed once daily with brine shrimp *Artemia* sp. (Bio-Marine; www.aquafauna.com) and Spirulina Flakes mixed with Freshwater Flake food (Ocean Star International Inc.; www.osishipping.com).

The experiment consisted of five treatments each replicated six times in 378.5 l outdoor mesocosms. Six males of different L_S were tested in each replicate: two small males (22–28 mm), two medium males (28.1–35 mm) and two large males (>35 mm). Testing males of different sizes mimicked more closely the composition of natural shoals and allowed for social interactions to occur. In each replicate, the six males were tested with 18 females to reproduce the 3:1 sex ratio that is found in natural populations (Hubbs, 1964). The treatments were as follows: (1) all *P. latipinna* females: 18 conspecific females (control 1), (2) high *P. latipinna* females: 15 *P. latipinna* females and three *P. formosa* females, (3) equal ratio: nine *P. latipinna* and nine *P. formosa* females, (4) high *P. formosa*: three *P. latipinna* females and 15 *P. formosa* females; and (5) all *P. formosa* females: 18 *P. formosa* females (control 2). By keeping female densities constant, but varying the frequencies of gynogenetic females, the effects of changing frequency of the gynogenetic species on the reproductive success of females of both species were analysed. A total of 540 females and 180 males were tested for a total of six replicates per treatment, but fewer individuals were included in the analyses because the last replicates of treatments 1 and 5 had to be discarded due to severe weather conditions that decimated the fish population in both trials. An attempt to match female sizes in treatments with both species was made, but sperm-dependent females were larger on average than host-species females (Table I). Fishes were maintained in the mesocosms for 28 days. A mesh screen was placed on top of the testing tanks to prevent predation. Fishes were fed once daily, and apart from feeding, they were left undisturbed until the conclusion of the testing period. Fishes were removed on the 28th day (the average brood cycle is 30 days; Constantz, 1989), to retrieve the embryos before the females dropped their broods, though this may have resulted in an underestimate of fertilization rates. This effect, however, should be spread equally across all treatments as the two species do not have significantly different brood cycles (Hubbs & Dries, 2002).

At the end of the experiment, females were euthanized using four drops of clove oil in 40 ml of water and dissected. The number of fertilized eggs as well as the proportion of fertilized females in each treatment was recorded. Females' L_S was measured both at the beginning (L_{si}) and at the end (L_{sf}) of the testing period.

TABLE I. Mean \pm S.E. female standard length (L_S) differences between species at the beginning (L_{si}) and the end (L_{sf}) of the testing period (ΔL_S , the change in L_S during the 28 days testing period). Wilcoxon rank-sum tests (Z) were performed whenever the variances associated with the measurements were significantly different between species, otherwise t -tests were performed

Treatment	<i>P. latipinna</i>	<i>P. formosa</i>	Z or t values	P
(2) High <i>Poecilia latipinna</i> females L_{si}	31.2 \pm 0.4	33.7 \pm 1.9	$Z = 1.38$	0.17
(2) High <i>P. latipinna</i> females L_{sf}	38.7 \pm 0.4	43.4 \pm 1.1	$t = -3.99$	<0.01*
(2) High <i>P. latipinna</i> females ΔL_S	8.13 \pm 0.8	8.15 \pm 1.6	$t = -0.01$	0.99
(3) Equal ratio L_{si}	29.4 \pm 0.8	33.7 \pm 1.4	$Z = 2.42$	0.01*
(3) Equal ratio L_{sf}	37.3 \pm 0.4	41.6 \pm 0.6	$Z = 5.74$	<0.001*
(3) Equal ratio ΔL_S	7.55 \pm 1.00	7.55 \pm 1.3	$t = -0.001$	0.99
(4) High <i>Poecilia formosa</i> L_{si}	29.9 \pm 0.6	38.1 \pm 1.2	$Z = -2.53$	<0.05*
(4) High <i>P. formosa</i> L_{sf}	38.4 \pm 1.0	43.3 \pm 0.3	$Z = -4.65$	<0.001*
(4) High <i>P. formosa</i> ΔL_S	6.82 \pm 2.0	5.34 \pm 1.2	$t = 0.63$	0.54
(1) All sexuals v. (5) all asexuals L_{si}	31.9 \pm 0.6	35.6 \pm 0.6	$t = -5.08$	<0.001*
(1) All sexuals v. (5) all asexuals L_{sf}	40.9 \pm 0.5	41.5 \pm 0.5	$t = -0.91$	0.30
(1) All sexuals v. (5) all asexuals ΔL_S	10.40 \pm 0.9	5.03 \pm 0.7	$t = 4.73$	<0.001*

*indicates significant P values.

STATISTICAL ANALYSES

To determine if the relative frequency of conspecific to heterospecific females affected the proportion of fertilized females of either species, a generalized linear model on the arcsine-transformed proportion of fertilized females with main effects of treatment, species and season was performed. The season effect was a nominal fixed effect that takes into consideration the time within the breeding season (early = March to May; middle = June to August; late = September to October). The control treatments (1 and 5) were excluded from this analysis. A Levene's test for equality of variances was performed including all treatments to determine if the variances around the mean proportion of fertilized females varied depending on the treatment. A Wilcoxon rank-sum test was performed between treatments 1 and 5 to test whether conspecific and heterospecific females were fertilized at different proportions when they were the only species of females available for males to mate.

To test whether the proportion of fertilized females in each treatment translated into different fitness consequences for either conspecific or heterospecific females, a mixed factor ANCOVA was performed on the number of fertilized eggs with female final L_S (L_{sf}) as a covariate and treatment, species and season as effects. A last set of analyses was conducted to compare the fecundity of the two species relative to each other and to the findings of Schlupp *et al.* (2010). The ratio of female *P. formosa* embryos to female *P. latipinna* embryos was calculated by dividing the total number of fertilized eggs for each female by the mother's L_{sf} to control for the fact that larger females produce more eggs and had more fertilized eggs (L_{sf} was a significant effect in the ANCOVA performed in the present study). The value obtained for *P. latipinna* was then divided by two to obtain the number of female embryos following Snelson & Wetherington (1980) who found that poeciliids show a 1:1 male:female embryo ratio. For *P. formosa*, the total number of fertilized eggs was only divided by the mother's L_{sf} because they only produce females. A Wilcoxon signed-rank test was used to compare the number of female embryos of each species.

RESULTS

The relative frequency of heterospecific to conspecific females did not have an effect on the proportion of females fertilized. There was no significant difference in the arcsine-transformed proportion of fertilized females between the treatments that had both female species present, whereas there was a significant effect of species and season (Table II). A significantly higher proportion of conspecific females were fertilized (*P. latipinna*: mean \pm s.e. = 0.65 ± 0.08 ; *P. formosa*: 0.35 ± 0.08 ; Wilcoxon signed rank, $W_{1,18} = 45.5$, $P < 0.001$). Although the time of the breeding season had a significant effect (Table II), the lack of a significant interaction with either

TABLE II. Effect tests for the generalized linear model on the proportion of fertilized females across treatments, seasons and species

Main effects	d.f.	χ^2	<i>P</i>
Treatment	2	0	1.00
Species	1	7.51	<0.01*
Season	2	9.32	<0.01*
Treatment \times species	2	3.04	0.24
Treatment \times season	4	4.52	0.34
Species \times season	2	0	1.00
Treatment \times season \times species	4	0.04	0.99

*indicates significant *P* values.

treatment or species suggests that both species were affected similarly. Finally, there was a significantly higher proportion of conspecific females with fertilized eggs when the two control treatments (1 and 5), where males had no choice of species, were compared (*P. latipinna*: mean \pm s.e. = 0.80 ± 0.03 , *P. formosa*: 0.34 ± 0.13 , Wilcoxon rank-sums $Z_{1,10} = 2.44$, $P < 0.01$).

Although the frequency of heterospecific gynogenetic females did not have an effect on the proportion of fertilized females of either species across treatments, it had an effect on the variance associated with the proportion of fertilized conspecific females. When the variances around the mean of each treatment (including 1 and 5) were compared for each species, there was a significant difference for the host species (Levene's test, $P < 0.001$) but not for the unisexual *P. formosa* (Levene's test, $P > 0.05$). The variance associated with treatments 1, 2 and 3 for *P. latipinna* (host) were significantly smaller than the variances associated with treatment 4 (high unisexual females; Fig. 1).

Male *P. latipinna* prefer to associate with larger females (Ptacek & Travis, 1997; Gabor, 1999), but a higher proportion of conspecific females were fertilized across treatments even though they were on average smaller than heterospecific *P. formosa* (Table I). Although an attempt to size match females at the beginning of the testing period was made, heterospecific females were significantly larger than conspecific females in all but treatment 1 (Table I). Larger females produce more eggs and have higher fitness (Travis *et al.*, 1990). Despite being smaller, however, *P. latipinna* had a larger number of fertilized eggs across all treatments (*P. latipinna*: mean \pm s.e. = 13.1 ± 0.8 ; *P. formosa*: 5.2 ± 0.6 ; Wilcoxon rank-sums, $Z_{1,489} = -8.3$; $P < 0.001$).

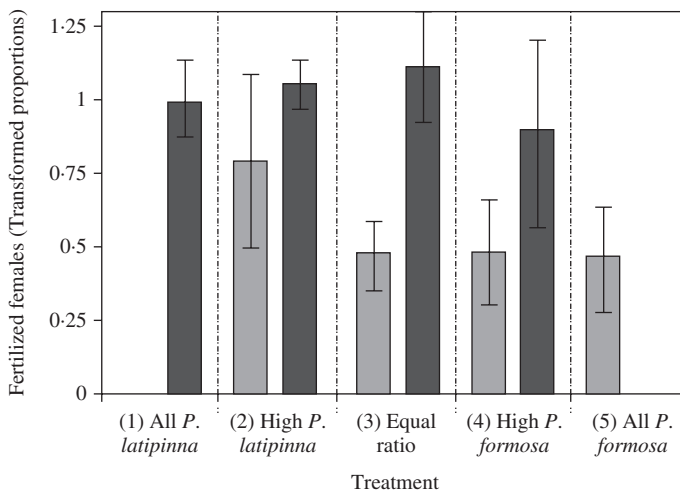


FIG. 1. Mean \pm s.e. proportion of fertilized females (arcsine transformed) by species [host species: *Poecilia latipinna* (■); sperm-dependent species *Poecilia formosa* (□)] within treatments. The treatments were as follows: (1) all *P. latipinna* females: 18 conspecific females (control 1), (2) high *P. latipinna* females: 15 *P. latipinna* females and three *P. formosa* females, (3) equal ratio: nine *P. latipinna* and nine *P. formosa* females, (4) high *P. formosa*: three *P. latipinna* females and 15 *P. formosa* females and (5) all *P. formosa* females: 18 *P. formosa* females (control 2). The variance associated with treatments (1), (2) and (3) for *P. latipinna* were significantly smaller than the variances associated with treatment (4) (Levene's test, $P < 0.01$).

TABLE III. Effect and covariate tests for ANCOVA on the number of fertilized eggs across treatments, species and seasons. Only treatments (2), (3) and (4) were used for this analysis

Main effects	d.f.	<i>F</i>	<i>P</i>
Species	1	11.30	<0.01*
Treatment	2	0.14	0.87
L_{sf}	1	3.96	<0.05*
Season	2	3.17	<0.05*
Species \times treatment	2	1.62	0.20
Species $\times L_{sf}$	1	4.00	<0.05*
Species \times season	2	2.13	0.12
Treatment $\times L_{sf}$	2	0.63	0.53
Treatment \times season	4	1.92	0.10
$L_{sf} \times$ season	2	1.49	0.23
Species \times treatment $\times L_{sf}$	2	0.67	0.51
Species \times treatment \times season	4	2.39	0.05
Species $\times L_{sf} \times$ season	2	0.11	0.89
Species \times treatment $\times L_{sf} \times$ season	4	0.27	0.89
Treatment $\times L_{sf} \times$ season	4	1.80	0.13

L_{sf} , standard length of the females at the end of the 28 day trials.

*indicates significant *P* values.

The ANCOVA performed using treatments 2, 3 and 4 indicated significant effects for the covariate L_{sf} , the main effects species and season, as well as a weak interaction between species and L_{sf} on the number of fertilized eggs per female (Table III). Given the lack of an interaction between season and species, no further analyses were performed. *Poecilia latipinna* produced more female embryos than *P. formosa* (*P. latipinna*: mean \pm S.E. = 0.1 ± 0.1 ; *P. formosa*: 0.12 ± 0.02 Wilcoxon rank-sums, $Z_{1,311} = -4.3$, $P < 0.001$).

DISCUSSION

Male *P. latipinna* fertilized a higher proportion of sexually reproducing conspecific females than gynogenetic heterospecific females across the breeding season regardless of the relative frequency of sexual to asexual females. The variance around the mean proportion of fertilized conspecific females across treatments increased as the number of unisexuals increased, suggesting that perhaps social interaction among males affects their mate choice. When conspecific females were abundant (treatments 1, 2 and 3; Fig. 1), there was little variance associated with the mean proportion of fertilized conspecific females and the variances were not statistically different between treatments, suggesting that males behaved similarly across the treatments. The variance for the mean number of fertilized conspecific females associated with treatment 4, when unisexuals were more abundant, was significantly larger than the ones associated with treatments 1, 2 and 3 for *P. latipinna* (Fig. 1). These results suggest that as the availability of conspecific females decreases and, therefore, male–male competition increases, then males are not as consistent in their mate preference as when conspecific females are abundant. The variances for

the mean proportion of fertilized unisexuals were not statistically different across treatments, suggesting that host-species males behaved similarly towards unisexuals regardless of their frequency.

Males may be more or less choosy depending on the context. When gynogenetic females constituted >50% of the female population *c.* 30% had developing embryos, while when they comprised 16% of the female population 50% had developing embryos. These results are in agreement with the findings of Moore & McKay (1971), who recorded that in natural populations of *Poeciliopsis* where unisexuals constituted >90% of the female population, only 5% were fertilized, whereas in populations where the unisexuals accounted for just 65% of the female population then 95% of them were fertilized. The results are also concordant with the findings of Heubel *et al.* (2009) who suggested that it might not always be beneficial for males to be choosy and discriminate against heterospecific matings despite the current population composition.

The preference for *P. latipinna* females was not exclusive, as several *P. formosa* were fertilized in most replicates. These mating events may have been due to the mistakes of smaller males, or unisexuals might have been inseminated after the males had mated with all available conspecific females. Differentiating between these two hypotheses is impossible with the data at hand. Additionally, when males were presented with only *P. formosa* (treatment 5), they mated with *c.* 30% of the females. Therefore, it can be inferred that, on average, at least *c.* 30% of heterospecific females are inseminated by male *P. latipinna* regardless of the population composition. Considering these results in the long term, 30% may still be enough inseminated females for the gynogenetic species to have enough offspring present in the next generation and explain the maintenance of this unisexual population. The results for treatment 5 support the claim by Hubbs (1964) that males attempt to court and mate with heterospecific females if conspecifics are not available. Nonetheless, it is obvious that such a situation in nature would not persist for long given the life span of the males and the fact that without the host males the sperm-dependent species cannot reproduce. A recent theoretical model, however, has shown how metapopulation dynamics with recurrent colonization events (Kokko *et al.*, 2008) can be sufficient to explain the maintenance of unisexual–bisexual mating complexes when local extinction of populations occurs. Therefore, male permissiveness may be playing an important role in the maintenance of the unisexual species.

Variation in mating behaviour among male *P. latipinna* across populations has been recorded (Ptacek & Travis, 1997; Gabor & Ryan, 2001; Gumm & Gabor, 2005; Gabor *et al.*, 2010) as well as seasonal plasticity (Heubel & Schlupp, 2008), but males from the specific population tested in the present study have shown a relatively constant preference for conspecific females. The preference for conspecific females was not lost even when heterospecific females were larger (Gumm & Gabor, 2005). Additionally, a preference for mid-sized females and not larger females was recorded for the males of the population used in the present study (Gabor *et al.*, 2010). These previous findings are supported with the present results. In the experimental treatments (2–4), growth was not different between species (Table I) and heterospecific females were larger than conspecific females at the end of all these treatments. *Poecilia formosa* were significantly larger than *P. latipinna* females but, despite this, no evidence was found of a species-mate quality recognition conflict. On the contrary, male *P. latipinna* fertilized more conspecific females

than heterospecific females in all treatments. These results, coupled with the results of previous work, suggest that perhaps males in this particular population have evolved a strong preference for conspecific females and have overcome the mate quality-species recognition conflict as previously suggested by Gumm & Gabor (2005).

Asexually reproducing organisms are predicted to have a two-fold reproductive advantage over sexually reproducing ones because they do not incur the cost of producing males (Maynard Smith, 1968). In the present study, this theoretical prediction was not supported. Not only was a higher proportion of conspecific females fertilized regardless of the frequency of the gynogenetic heterospecific females but also a higher number of conspecific eggs. Sexually reproducing *P. latipinna* females on average had a higher number of fertilized eggs than gynogenetic *P. formosa*. *Poecilia formosa* did not have a two-fold reproductive advantage over host-species females based on the number of female embryos produced per female whereas Schlupp *et al.* (2010) found the opposite results. The results reported here are more in agreement with the findings of Riesch *et al.* (2008).

Both the present results and Riesch *et al.* (2008) suggest that host-species males inseminate more conspecific females than heterospecific ones. Heubel & Schlupp (2008) did not find support for this conclusion. Although Heubel & Schlupp (2008) did not find evidence for any frequency-dependent regulating mechanisms, which comports with the present study, Heubel & Schlupp (2008) found a significant interaction between season and species on the association preference of host-species males, whereas no effect of an interaction on the proportion of fertilized females of either species was detected in the present study. The present results may be different from those of Heubel & Schlupp (2008) because association preference in a controlled setting was not the response variable of interest, instead male preference was extrapolated from the proportion of females they inseminated and fertilized in both species in a mesocosm setting. Overall, in the present study, the presence of the sperm parasite *P. formosa* does not appear to have a two-fold advantage over the host species and male *P. latipinna* show a clear preference for conspecific over heterospecific females independent of season.

The frequency of unisexual sperm-dependent individuals varies in time and space within a unisexual–bisexual mating system (Hubbs, 1964; Booi & Guldmond, 1984; Vrijenhoek, 1994; Heubel *et al.*, 2009), but although population dynamics were not tested over an extensive period of time, the present findings may still have a long-term predictive significance. The lack of frequency-dependent mating preference on the part of males and the constant higher fecundity of host-species females regardless of the frequency of heterospecific unisexual females suggest that this variation in frequency over time is most probably due to ecological mechanisms such as lower tolerance to stressful food or temperature conditions or competition with the host species for resources (Alberici da Barbiano *et al.*, 2010; Tobler & Schlupp, 2010).

Thanks to Sigma Xi Student research Grant awarded to L.A.B. and NSF-IOB-1021873 awarded to C.R.G., A.S.A. and C.C.N. We thank the Mexican Government for collection permit # 4777-010705-9522. We thank C. Espinedo and K. Epp for logistical support, the GASP laboratory students for fish maintenance. M. Ptacek and the EEB discussion group for providing helpful insight. Fishes were euthanized following protocols of accepted IACUC # 06-0621_802.

References

- Alberici da Barbiano, L., Waller, J. & Gabor, C. R. (2010). Differences in competitive efficiency between a sexual parasite and its host provide insight into the maintenance of a sperm-dependent vertebrate species. *Journal of Freshwater Ecology* **25**, 523–530.
- Alonzo, S. H. & Sinervo, B. (2001). Mate choice games, context-dependent good genes, and genetic cycles in the side-blotched lizard, *Uta stansburiana*. *Behavioral Ecology and Sociobiology* **49**, 176–186.
- Avise, J. C., Trexler, J. C., Travis, J. & Nelson, W. (1991). *Poecilia mexicana* is the recent female parent of the unisexual fish *P. formosa*. *Evolution* **45**, 1530–1533.
- Baird, R. C. (1968). Aggressive behavior and social organization in *Mollinesia latipinna* La Seuer. *Texas Journal of Science* **20**, 157–176.
- Booij, C. J. H. & Guldemond, J. A. (1984). Distributional and ecological differentiation between asexual gynogenetic planthoppers and related sexual species of the genus *Muellerianella* (Homoptera, Delphacidae). *Evolution* **38**, 163–175.
- Constantz, G. D. (1989). Reproductive biology of poeciliid fishes. In *Ecology and Evolution of Livebearing Fishes* (Poeciliidae) (Meffe, G. K. & Snelson, F. F., eds), pp. 91–123. Upper Saddle River, NJ: Prentice Hall.
- Coyne, J. A. & Orr, H. A. (2004). *Speciation*. Sunderland, MA: Sinauer Associates.
- Dawley, R. M. & Bogart, J. P. (Eds) (1989). Evolution and ecology of unisexual vertebrates. *Bulletin of the New York State Museum* **466**.
- Dries, L. A. (2003). Peering through the looking glass at a sexual parasite: are Amazon mollies red queens? *Evolution* **57**, 1387–1396.
- Farr, J. A. (1989). Sexual selection and secondary sexual differentiation in the Poeciliidae: determinants of male mating success and the evolution of female choice. In *Ecology and Evolution of Livebearing Fishes* (Poeciliidae) (Meffe, G. K. & Snelson, F. F., eds), pp. 91–123. Upper Saddle River, NJ: Prentice Hall.
- Gabor, C. R. (1999). Association patterns of sailfin mollies (*Poecilia latipinna*): alternative hypotheses. *Behavioral Ecology and Sociobiology* **46**, 333–340.
- Gabor, C. R. & Ryan, M. J. (2001). Geographical variation in reproductive character displacement in mate choice by male sailfin mollies. *Proceedings of the Royal Society B* **268**, 1063–1070.
- Gabor, C. R., Gonzalez, R., Parmley, M. & Aspbury, A. S. (2010). Variation in male sailfin molly preference for female size: does sympatry with sexual parasites drive preference for smaller conspecifics? *Behavioral Ecology and Sociobiology* **64**, 783–792.
- Graf, J. & Polls Pelaz, M. (1989). Evolutionary genetics of the *Rana obscurulenta* complex. In *Evolution and Ecology of Unisexual Vertebrates* (Dawley, R. M. & Bogart, J. P., eds), pp. 289–302. Albany, NY: New York State Museum.
- Gumm, J. M. & Gabor, C. R. (2005). Asexuals looking for sex: conflict between species and mate-quality recognition in sailfin mollies (*Poecilia latipinna*). *Behavioral Ecology and Sociobiology* **58**, 558–565.
- Heubel, K. U. & Schlupp, I. (2008). Seasonal plasticity in male mating preferences in sailfin mollies. *Behavioral Ecology* **19**, 1080–1086.
- Heubel, K. U., Rankin, D. J. & Kokko, H. (2009). How to go extinct by mating too much: population consequences of male mate choice and efficiency in a sexual-aseual species complex. *Oikos* **118**, 513–520.
- Hubbs, C. (1964). Interactions between bisexual fish species and its gynogenetic sexual parasite. *Bulletin of the Texas Memorial Museum* **8**, 1–72.
- Hubbs, C. & Dries, L. A. (2002). Geographic variation in interbrood interval in *Poecilia*. In *Libro jubilar en honor al Dr. Salvador Contreras Balderas* (Lozano-Vilano, L., ed.), pp. 35–41. San Nicolás de los Garza: Universidad Autónoma de Nuevo Leon.
- Kawecki, T. J. (1988). Unisexual bisexual breeding complexes in Poeciliidae: why do males copulate with unisexual females? *Evolution* **42**, 1018–1023.
- Kokko, H., Heubel, K. U. & Rankin, D. J. (2008). How populations persist when asexuality requires sex: the spatial dynamics of coping with sperm parasites. *Proceedings of the Royal Society B* **275**, 817–825.
- Maynard Smith, J. (1968). Evolution in sexual and asexual populations. *American Naturalist* **102**, 469–473.

- Marler, C. A. & Ryan, M. J. (1997). Origin and maintenance of a female mating preference. *Evolution* **51**, 1244–1248.
- Moore, W. S. & McKay, F. E. (1971). Coexistence in unisexual species complexes of *Poeciliopsis* (Pisces-Poeciliidae). *Ecology* **52**, 791–799.
- Pfennig, K. S. (1998). The evolution of mate choice and the potential for conflict between species and mate-quality recognition. *Proceedings of the Royal Society B* **265**, 1743–1748.
- Plath, M., Blum, D., Schlupp, I. & Tiedemann, R. (2008). Audience effect alters mating preferences in a livebearing fish, the Atlantic molly, *Poecilia mexicana*. *Animal Behavior* **75**, 21–29.
- Ptacek, M. B. & Travis, J. (1997). Mate choice in the sailfin molly, *Poecilia latipinna*. *Evolution* **51**, 1217–1231.
- Riesch, R., Schlupp, I. & Plath, M. (2008). Female sperm limitation in natural populations of a sexual/asexual mating complex (*Poecilia latipinna*, *Poecilia formosa*). *Biology Letters* **4**, 266–269.
- Ryan, M. J. & Rand, A. S. (1993). Species recognition and sexual selection as a unitary problem in animal communication. *Evolution* **47**, 647–657.
- Ryan, M. J., Dries, L. A., Batra, P. & Hillis, D. M. (1996). Male mate preference in a gynogenetic species complex of Amazon mollies. *Animal Behavior* **52**, 1225–1236.
- Schartl, M., Nanda, I., Schlupp, I., Wilde, B., Epplen, J. T., Schmid, M. & Parzefall, J. (1995a). Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish. *Nature* **373**, 68–71.
- Schartl, M., Wilde, B., Schlupp, I. & Parzefall, J. (1995b). Evolutionary origin of a parthenoform, the Amazon molly, *Poecilia formosa*, on the basis of a molecular genealogy. *Evolution* **49**, 827–835.
- Schlupp, I., Marler, C. & Ryan, M. J. (1994). Benefit to male sailfin mollies of mating with heterospecific females. *Science* **263**, 373–374.
- Schlupp, I., Taebel-Hellwig, A. & Tobler, M. (2010). Equal fecundity in asexual and sexual mollies (*Poecilia*). *Environmental Biology of Fishes* **88**, 201–206.
- Schmeller, D. S., O'Hara, R. & Kokko, H. (2005). Male adaptive stupidity: male mating pattern in hybridogenetic frogs. *Evolutionary Ecology Research* **7**, 1039–1050.
- Snelson, F. F. & Wetherington, J. D. (1980). Sex ratio in the sailfin molly, *Poecilia latipinna*. *Evolution* **34**, 308–319.
- Tobler, M. & Schlupp, I. (2010). Differential susceptibility to food stress in neonates of sexual and asexual mollies (*Poecilia*, Poeciliidae). *Evolutionary Ecology* **24**, 39–47.
- Travis, J. & Woodward, B. D. (1989). Social context and courtship flexibility in male sailfin mollies, *Poecilia latipinna* (Pisces: Poeciliidae). *Animal Behavior* **38**, 1001–1011.
- Travis, J., Trexler, J. C. & Mulvey, M. (1990). Multiple paternity and its correlates in female *Poecilia latipinna* (Poeciliidae). *Copeia* **1990**, 722–729.
- Vrijenhoek, R. C. (1994). Unisexual fish – model systems for studying ecology and evolution. *Annual Review of Ecology and Systematics* **25**, 71–96.
- Woodhead, A. D. & Armstrong, N. (1985). Aspects of mating behaviour of male mollies. *Journal of Fish Biology* **27**, 593–601.