Mixed-species shoals and the maintenance of a sexual–asexual mating system in mollies

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Abstract. The mating system of the sexual–asexual complex of sailfin mollies, *Poecilia latipinna*, and Amazon mollies, *P. formosa*, depends on the close proximity of individuals in aggregations or shoals because *P. formosa* is gynogenetic (that is, sperm only initiate embryogenesis but do not contribute genetic material) and must be inseminated by male *P. latipinna*. Furthermore, female *P. latipinna* copy the mate choice of female *P. formosa* with the result that male *P. latipinna* become more attractive to conspecific females when they mate with *P. formosa*. Mate copying of heterospecifics has not been reported in other systems, and in mollies it could result from the inability of females to discriminate species. In experiments both *P. formosa* and *P. latipinna* females preferred to associate with a conspecific than a heterospecific, but preferred a larger heterospecific group to a smaller conspecific one. Females can thus discriminate between conspecific and heterospecific females, so that heterospecific mate copying is not a result of mistaken identity. Females preferred to associate with conspecifics, but this preference was overridden by preference for larger groups although the threshold differed between the two species.

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The Amazon molly, *Poecilia formosa* (Girard 1859), is an all-female species of fish of hybrid origin (Hubbs & Hubbs 1932; Schartl et al. 1995b) which relies on insemination with sperm from males of closely related sexual species (Turner 1982; Balsano et al. 1989). It reproduces by gynogenesis, that is the sperm normally only trigger embryogenesis without a genetic contribution (but see Schartl et al. 1995a). The geographical range of *P. formosa* encompasses two sexual species, *P. mexicana* Steindachner 1863, which is widely distributed throughout the Atlantic regions of Mexico, and *P. latipinna* (Le Sueur 1821), which is found in northeastern Mexico, southeastern Texas, and in an introduced population in the San Marcos River in central Texas (Courtenay & Meffe 1989). In Mexico *P. formosa* generally live

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in the same habitat and in mixed shoals with *P. mexicana*, and in Texas with *P. latipinna*. *Poecilia mexicana* is known to be the maternal ancestor of this hybrid (Avise et al. 1991), and recent research (Schartl et al. 1995b) shows that either an unknown population of *P. latipinna* or an extinct form from the *P. latipinna* ancestry was the paternal form. This group has received considerable interest from evolutionary biologists because the maintenance of this mating complex depends upon mixed-species associations and mating between heterospecifics.

It is difficult to understand why host males mate with female *P. formosa* since this should involve some costs and no apparent benefits for the males. It is not due to the males being unable to discriminate. Several studies have shown that both *P. mexicana* males (Schlupp et al. 1991) and *P. latipinna* males (Hubbs 1964; Ryan et al., in press; I. Schlupp, M. Schartl & J. Parzefall, unpublished data) can do so. Mate copying (e.g. Dugatkin 1992) might offer a resolution (Schlupp et al. 1994). Female *P. latipinna* copy the mate choice of *P. formosa*. Thus male *P. latipinna*

become more attractive to their own females by mating with *P. formosa*. The *P. formosa–P. latipinna* system is the only known case of heterospecific mate copying.

The necessity of both mixed-species shoals and heterospecific mating to maintain this sexual–asexual species complex raises some obvious questions about discrimination, preference of association and group size. So far, no adaptive advantage of mate copying to the copying individual has been clearly demonstrated. However, some hypotheses have been suggested, such that young females copy older ones because of the latter's experience in choosing better males (Dugatkin & Godin 1993). It is possible, however, that copying in mollies results from misidentification. Thus we determined if female *P. latipinna* can discriminate between conspecific and *P. formosa* females. Many species prefer to associate with conspecifics, a phenomenon known as conspecific cueing (Kiester 1979; Stamps 1988; Reed & Dobson 1993). We tested these association patterns among females of *P. latipinna* and *P. formosa*. A preference for associating with conspecifics would reject the null hypothesis that female *P. latipinna* are not able to discriminate between conspecific and *P. formosa* females. If female *P. latipinna* were to prefer to associate with conspecifics rather than with female *P. formosa*, that is, if they could discriminate between species, this raises questions about the maintenance of mixed-species shoals.

Conspecific cueing can result in social aggregations with inherent benefits. The presence of conspecifics might, for example, indicate suitable habitat, potential mates, food sources or relative safety (Kiester 1979; Stamps 1988; Reed & Dobson 1993; Avery 1994). In this case the advantage arises from the fact that the conspecifics indicate some relevant information. Other benefits depend more on group size than the specific make-up of the group. An example is the selfish herd effect as a deterrence against predation (Hamilton 1971), an effect especially well documented in fish (Pitcher & Parrish 1993).

Therefore, we conducted a series of experiments to determine if a preference for conspecific associations would be overridden by a preference for associating with larger, heterospecific groups. Such an effect would be consistent with the observations of mixed-species schools of *P. formosa* and *P. latipinna*, and would contribute towards the maintenance of this sexual–asexual complex.

METHODS

All fish were collected in 1993 from a population in central Texas (Martindale, San Marcos River) and maintained in stock tanks in the laboratory or in a large outdoor breeding tank under natural conditions in the Brackenridge Field Laboratory, University of Texas. In the laboratory the fish were fed commercially available flake food and maintained on a 12:12 h artificial light:dark cycle that supplemented natural light and a temperature of about 25°C. After the experiments the fish were returned to the stock tanks.

We gave females of *P. latipinna* and *P. formosa* a choice between one conspecific and either one, two, or three heterospecific females. In controls, all the fish were conspecific. These females were matched for size (± 1 mm standard length). Each female was used as a test fish only once. The experimental tank ($122 \times 32 \times 52$ cm) was divided into five sections. The compartments on each side were partitioned from the rest of the tank by Plexiglas dividers. Thus water flow and chemical communication between them was absent or at least very limited. The central compartment was divided into three sections by markings drawn on the sides and bottom of the tank. Two sections were adjacent to another female in the two end compartments and the middle section was defined as a neutral zone. The test female was able to move freely between these three central sections.

After an acclimation period of 20 min, we observed the females for 10 min and recorded the time spent in each of the three sections. The stimulus females were then switched between the end compartments of the tank and the experiment was repeated to control for a potential side bias. We decided a priori that a side bias occurred when a female spent more than 80% of her time on the same side for both trials; such trials were not included in the analysis. We used the same experimental tank and conditions as described in Schlupp et al. (1994). Percentages were calculated from the time spent in the sections adjacent to a female; the time in the neutral zone was not counted.

We calculated a response index as the percentage of the total 20-min observation period that
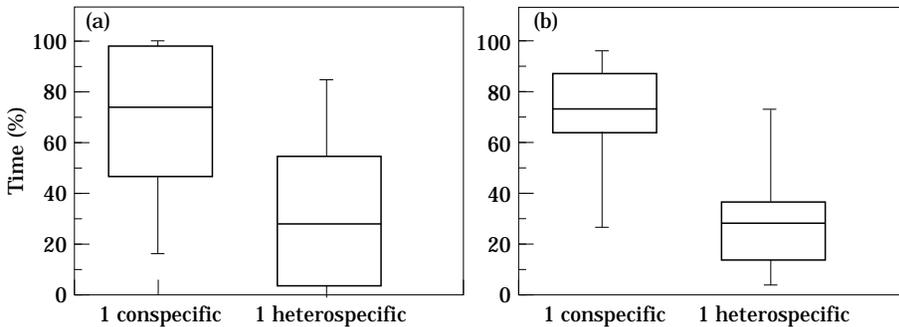
Figure 1. The percentage of time that (a) *P. latipinna* and (b) *P. formosa* spent with either one conspecific or one heterospecific when allowed to choose between them. The line in the box indicates the median, the lower and upper ends of the box the 25% and 75% values and the two whiskers the 10% and 90% values, respectively.

females spent outside the neutral zone, that is adjacent to another female. The mean overall response index was 91.42% ($\pm$ 8.56% SD), indicating that the females were actively choosing between the offered alternatives.

All *P*-values are two-tailed.

RESULTS

One Heterospecific Versus One Conspecific

Both *P. latipinna* and *P. formosa* preferred conspecific females when given a choice between one conspecific and one heterospecific (Wilcoxon tests: *P. latipinna*: $z = -2.091$, $N=20$, $P=0.036$; *P. formosa*: $z = -2.438$, $N=17$, $P=0.015$; Fig. 1). As a control, we gave females a choice between two conspecifics and found no preference (Wilcoxon tests: *P. latipinna*: $z = -0.723$, $N=7$, $P=0.47$; *P. formosa*: $z=0.169$, $N=7$, $P=0.17$).

Two Heterospecifics Versus One Conspecific

When given a choice between two heterospecifics and a conspecific, *P. formosa* still preferred conspecifics (Wilcoxon test: $z = -2.31$, $N=9$, $P=0.021$), whereas *P. latipinna* showed no preference (Wilcoxon test: $z = -0.284$, $N=15$, $P=0.78$). In the respective controls, in which all test fish were conspecifics, both forms showed a clear preference for the shoal (Wilcoxon tests: *P. latipinna*: $z = -2.103$, $N=8$, $P=0.035$; *P. formosa*: $z = -2.21$, $N=9$, $P=0.027$; Fig. 2).

The tendency to be with the conspecific is significantly different between the two species (Welch's *t*-test: $t=2.13$, $df=21$, $P<0.05$).

Three Heterospecifics Versus One Conspecific

When given a choice between three heterospecifics and a conspecific both types of female preferred the shoal (Wilcoxon tests: *P. latipinna*: $z = -2.384$, $N=7$, $P=0.017$; *P. formosa*: $z = -2.366$, $N=7$, $P=0.018$; Fig. 3).

DISCUSSION

Both *P. latipinna* and *P. formosa* strongly preferred to associate with a conspecific female rather than a heterospecific when there was one of each. When there were two heterospecifics and one conspecific a trade-off between shoaling and conspecific cueing became apparent and each species had a different threshold for shifting from conspecific cueing to shoaling. When there were three heterospecifics and one conspecific shoaling behaviour became prevalent. These results suggest that larger shoals might confer some advantage for the females; the exact nature of this advantage was not investigated.

Our results indicate that both *P. formosa* and *P. latipinna* females recognize the two species. Thus the hypothesis that *P. latipinna* females copy the mate choice of *P. formosa* because they cannot distinguish between the two types of female can be rejected.

These results raise the question why *P. latipinna* females would be willing to copy the mate preference of the heterospecific *P. formosa*. If mate copying is adaptive, and we emphasize there is still no good evidence suggesting this is so, then we assume that, by copying, females gain some information from the model, such as the presence of an appropriate mate. Thus, female *P. formosa* might convey this information as reliably as *P. latipinna*.
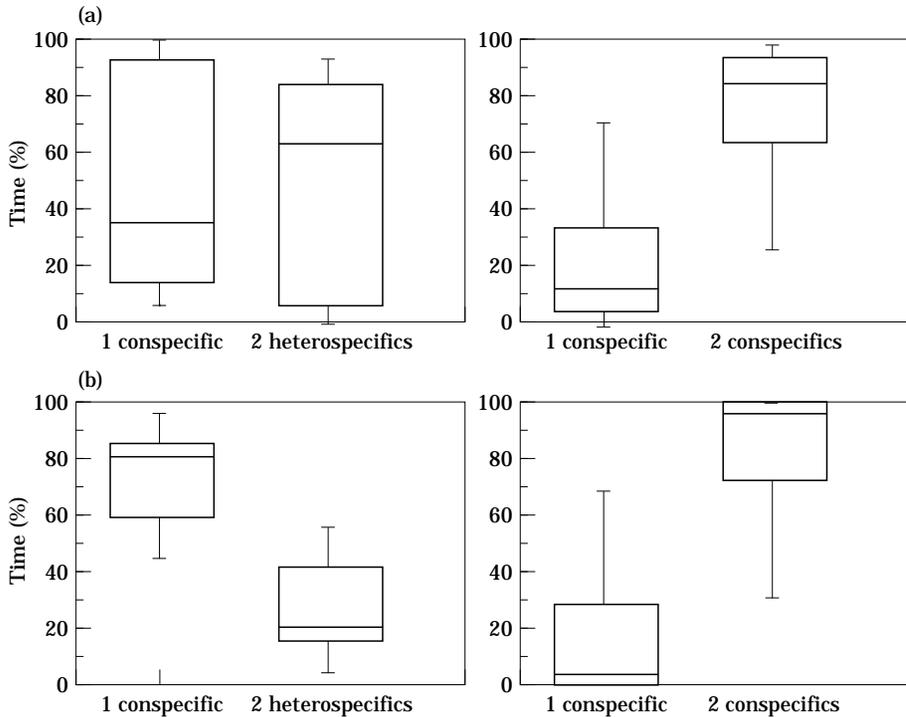
Figure 2. The percentage of time that (a) *P. latipinna* and (b) *P. formosa* spent with either two heterospecifics and a conspecific or two conspecifics when allowed to choose between them. The line in the box indicates the median, the lower and upper ends of the box the 25% and 75% values and the two whiskers the 10% and 90% values, respectively.
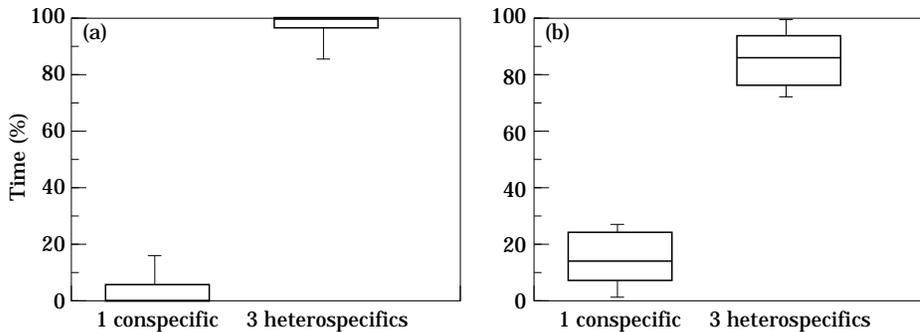

Figure 3. The percentage of time that (a) *P. latipinna* and (b) *P. formosa* spent with either three heterospecifics or one conspecific when allowed to choose between them. The line in the box indicates the median, the lower and upper ends of the box the 25% and 75% values and the two whiskers the 10% and 90% values, respectively.

Females of both species mate with the same males, and C. Marler & M. J. Ryan (personal communication) have shown that both *P. formosa* and *P. latipinna* females have the same preference with respect to male body size. Furthermore, females compete with each other for access to these males (Schlupp et al. 1991; Foran & Ryan 1994).

The degree of aggressive interactions between females of *P. latipinna* and *P. formosa* for access to males might be another peculiar feature of this mating system. To our knowledge no other studies of poeciliid mating systems have revealed such high levels of female competition over access to males. In a different context, female guppies,

Poecilia reticulata, compete for other resources such as food (Magurran & Seghers 1994).

Both *P. formosa* and *P. latipinna* experience more aggression from heterospecific females than from conspecifics (Foran & Ryan 1994). Less aggression from a conspecific will probably facilitate conspecific shoals. In the presence of males, *P. latipinna* females are more aggressive towards *P. formosa* than towards *P. latipinna* females. Therefore, *P. formosa* may be avoiding aggression by switching to heterospecific shoals later than *P. latipinna*.

Kin selection may help to explain why the clonal *P. formosa* show less aggression to conspecifics than to heterospecifics. Based on tissue grafts, Kallman (1962) estimated that only two *P. formosa* clones exist in the San Marcos River. This is supported by DNA fingerprinting analysis with a small number (7) of specimens, which detected no more than two clones (M. Schartl, B. Wilde, J. T. Epplen, I. Schlupp & J. Parzefall, unpublished data). A related argument may apply to *P. latipinna*: among themselves they are more related than with any *P. formosa*, even though Simanek (1978) reported a very high degree of genetic variability ($H = 10$) for this particular *P. latipinna* population. In any case this is another factor that would favour conspecific shoals and thus appears to be overridden by the larger number of heterospecifics.

In summary, our results can explain the apparent paradox that *P. latipinna* and *P. formosa* can form mixed shoals despite a strong preference for conspecifics, and this shoaling behaviour facilitates the maintenance of the *P. formosa*–*P. latipinna* mating system.

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