

Prudent male mate choice under perceived sperm competition risk in the eastern mosquito fish

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In contrast to what is known about adaptive mate choice in females, we know far less about how fluctuating reproductive costs might affect male mate choice. In many species, sperm competition can have a direct bearing on male fertilization success, and choosy males should be expected to respond adaptively to the perceived cost of sperm competition and to adjust their mate preferences accordingly. Here, we conducted a series of experiments investigating male mate choice under sperm competition risk in the eastern mosquito fish, *Gambusia holbrooki*. We tested male association preferences before and after manipulating their perceptions of sperm competition risk associated with initially preferred and nonpreferred females. We found that individuals were consistent in their preferences if they did not have the opportunity to witness other males associating with the initially preferred female. By contrast, males spent significantly less time with initially preferred females if, in the interim, she had been seen in the vicinity of another male. A similar opportunity to observe the initially nonpreferred female with another male had no effect on subsequent male mate choice. Our results suggest that choosy males may be capable of adjusting their preferences in response to shifts in their perception of sperm competition risk. *Key words*: eavesdropping, intrasexual competition, mate choice copying, Poeciliidae, sexual selection. [*Behav Ecol* 20:278–282 (2009)]

Male reproduction can be a costly endeavor. In many species, males typically search for females, and this may require a substantial investment of both time and resources (Kokko and Wong 2007). In addition, male courtship displays can be energetically demanding to perform (Kotiaho et al. 1998; Judge and Brooks 2001), nuptial gifts can be taxing to produce (Engqvist and Sauer 2001), and male reproductive attempts can sometimes culminate in serious injuries (Stuart-Fox and Whiting 2005) or even death (Gaskett et al. 2004; Kasumovic et al. 2007). With the production of ejaculates generating nontrivial costs, the availability of sperm can also impose a severe limit on male reproductive opportunities (Dewsbury 1982; Bonduriansky 2001). Given that females often vary greatly in reproductive value, males are expected to engage in strategies that can increase their fertilization success in order to maximize their reproductive payoffs (Galvani and Johnstone 1998; Heubel and Schlupp 2008).

Evidence suggests that male mating effort can be influenced by both the risk and intensity of sperm competition (Wedell et al. 2002; Thomas and Simmons 2007). Sperm competition describes the situation that arises when the sperm of different males compete to fertilize a female's eggs (Parker 1970). In species with internal fertilization, such competition can occur whenever a female mates with multiple suitors, and live sperm of different males can be present simultaneously within her reproductive tract. Most theoretical and empirical studies examining adaptive male responses to sperm competition tend to focus on the final stages of reproduction (reviewed in Wedell et al. 2002). Based on these, it is now well accepted that males, during mating, are capable of modulating both the size and quality

of their ejaculates in response to sperm competition risk (Candolin and Reynolds 2002; Pilastro et al. 2002; Zbinden et al. 2003; Kilgallon and Simmons 2005; Aspbury 2007; Simmons et al. 2007). Precopulatory behavioral responses, such as male mate choice, are also likely to be important (Dosen and Montgomerie 2004; Schlupp and Plath 2005; Plath, Blum, et al. 2008; Plath, Richter, et al. 2008). For male mate choice to be adaptive, choosy individuals need be sensitive to changes in sperm competition risk and to be able to adjust their mating preferences accordingly. Yet, in contrast to what is known about adaptive mate choice in females, we know far less about how fluctuating reproductive costs might affect choosy males (Wong and Jennions 2003; Gaskett et al. 2004; Byrne and Rice 2006).

The eastern mosquito fish, *Gambusia holbrooki*, is an ideal study species with which to investigate the effects of perceived sperm competition risk on male mate choice. Like other poeciliid fishes, fertilization in this species takes place internally. During copulation, males transfer bundles of sperm to the female by using their modified anal fin, the gonopodium, as an intromittent organ (Constantz 1989). In contrast to some poeciliids, male eastern mosquito fish do not use elaborate courtship displays to solicit copulations but attempt forced copulations by thrusting their gonopodia toward the female's genital pore (Bisazza 1993; Bisazza and Marin 1995). Sperm competition is likely to be intense in this species due to high levels of multiple paternity within broods (Zane et al. 1999), as well as the ability of females to store sperm (Constantz 1984) and to exert some control over the success of male mating attempts (Bisazza et al. 2001). Males, in this regard, have been shown to increase their sperm expenditure under heightened risk of sperm competition (Evans et al. 2003). But how does sperm competition risk affect a male's choice of potential mating partner in the first place? Here, we experimentally investigate whether male mosquito fish adjust their preferences in response to changes in the perception of sperm competition risk associated with initially preferred and nonpreferred females.

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METHODS

Animal collection and housing

Mosquito fish used in our study were sexually mature individuals collected before the start of the breeding season in early September from an introduced population in southeastern Australia. The maximum life span of fish from this region is typically less than 15 months (Cadwallader and Backhouse 1983). Adult sex ratios in the wild are usually female biased early in the season (due to differential survival of males and females over winter) but become increasingly male biased over time (Zulian et al. 1995). Back in the laboratory, the sexes were housed separately for a minimum of 4 weeks prior to testing to try and maximize sexual responsiveness and to confirm that none of the females were pregnant before the start of the study. Adult males can be distinguished from females by the presence of a fully developed gonopodium (modified anal fin) and the absence of a gravid spot on their abdomens (reviewed in Pyke 2005). During the holding period, fish were kept in several 50-l aquaria on a 12:12 h light:dark cycle and fed on an ad libitum diet of commercially manufactured fish flakes. Water quality was maintained through the use of external power filters and weekly partial water changes.

Experimental procedure

Experimental trials consisted of 3 distinct stages (Figure 1). In stage 1, we measured the association preferences of male mosquito fish (mean standard length [SL] \pm standard deviation [SD] = 17.48 \pm 1.74 mm) when presented with a dichotomous choice between 2 females randomly selected from a stock tank and assigned to 1 of 2 small aquaria (length \times width = 30 \times 15 cm) placed lengthwise at opposite ends of

a larger aquarium (60 \times 30 cm) housing the focal male. All aquaria were filled with water to a depth of 10 cm. A sheet of white paper inserted between the large aquarium and each of the 2 smaller aquaria prevented the fish from seeing one another. After a 10-min acclimation period, we gently lifted both sheets and recorded the amount of time the male spent associating with each of the females during a 10-min observation period. The male was deemed to be associating with a particular female if he was within 5 cm of her aquarium, with his body oriented unambiguously toward her. At the completion of stage 1, the proportion of time spent with the preferred female was calculated from the total time spent associating with both females [i.e., time with preferred female / (time with preferred female + time with unpreferred female)]. The "preferred" female was determined to be the one with whom the focal male had spent the most time in association (Wong and Jennions 2003). We terminated any trials at this stage if focal males spent equal time with both females (resulting in one of the trials being excluded in experiment 3). Association time is widely used in studies of mate choice in fishes and is the standard measure of male mating preferences in poeciliids (e.g., Schlupp and Ryan 1997; Dosen and Montgomerie 2004; Wong et al. 2005). In the wild, male mosquito fish closely associate with females during the breeding season (Martin 1975) and, due to their coercive mating system, must be in close proximity before any insemination attempts can be made (Bisazza et al. 2001). In our trials, male proximity to stimulus females was often accompanied by flexing of the gonopodium, which is a characteristic sexual response associated with male mating attempts (Pyke 2005). Association time was therefore taken as a biologically meaningful estimate of male mating preferences. The SL (\pm SD) of preferred and unpreferred females was 23.31 \pm 0.75 and 15.89 \pm 0.69 mm, respectively, with the former being significantly larger than the latter (paired *t*-test: *t* = 2.88, *P* = 0.006).

During stage 2, the focal male was allowed to observe both females for 30 min, either alone or in the vicinity of another male. To do so, the focal male was gently netted and transferred into a clear perspex cylinder located in the center of the large aquarium. Each of the 2 smaller aquaria was then divided into half with a clear plastic divider, confining each female to one half of her aquarium whereas a stimulus male was introduced into the other half. Stimulus males (SL \pm SD = 17.4 \pm 1.8 mm) were randomly selected (from a separate stock tank to the one housing focal males) and assigned to the female compartments. The use of opaque partitions allowed us to manipulate the ability of the focal male to observe, depending on the experiment (see below), either one or both of the stimulus males associating with the stimulus females. Allowing a stimulus male to associate with each female (but avoiding physical contact) ensured that both females were subjected to the same social conditions to avoid any potential differences in female experience and/or recent mating history from affecting their subsequent attractiveness in stage 3 (Bisazza et al. 1989). There was no difference in body size between stimulus and focal males (*t* = 0.18, *P* = 0.85).

During stage 3, we reinserted the white sheets separating the main tank and the 2 smaller aquaria. We then removed the stimulus males and the clear dividers in the 2 smaller aquaria and released the focal male from the perspex cylinder into the main tank. After a 10-min acclimation period, we gently lifted the sheets so that the focal male could, once again, see and interact with both females. We then rerecorded the amount of time he spent associating with each female during a 10-min observation period. From this, we were able to compare the proportion of time spent with the initially preferred

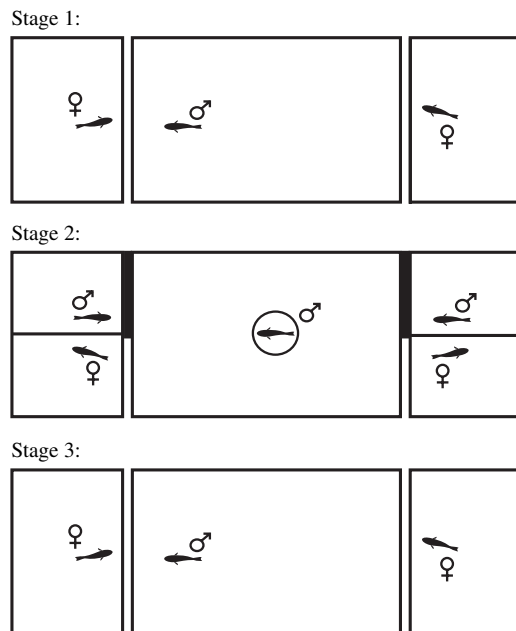


Figure 1
Experimental setup. Male association preferences was measured before (stage 1) and after (stage 3) manipulating male perception of sperm competition risk (stage 2). The latter was achieved by isolating focal males in a perspex cylinder and using carefully placed partitions (black bars) to influence, depending on the experiment, their ability to see other males associating with initially preferred and nonpreferred females.

female in stages 1 and 3. Recent studies on other poeciliids have shown that male mating preferences can be influenced by the presence of rivals (Schlupp and Plath 2005; Plath, Blum, et al. 2008; Plath, Richter, et al. 2008). Removing the stimulus males prior to retesting was therefore necessary to control for any potential confounds arising from these so-called “audience effects” (sensu Plath, Blum, et al. 2008).

Using the setup described above, we conducted 3 separate experiments (see below).

Experiment 1: male choice in the absence of perceived sperm competition risk

In this experiment, we tested the repeatability of male association preferences in the absence of perceived sperm competition risk ($N = 15$). Here, opaque partitions were placed in front of the compartments of both stimulus males during stage 2 so that the focal male could not directly see any other males in the vicinity of the stimulus females. Because focal males in this experiment did not have the opportunity to directly observe a stimulus male associating with either of the females, we predicted that the time spent by the focal male in association with the initially preferred female in stage 1 should not differ from that in stage 3.

Experiment 2: male choice in the presence of perceived sperm competition risk

In this experiment, we tested the repeatability of male mate choice under perceived sperm competition risk ($N = 15$). Here, during stage 2, the focal male was able to see the stimulus male adjacent to the compartment of the initially preferred female. An opaque partition, however, prevented the focal male from seeing the stimulus male that was adjacent to the initially nonpreferred female. This setup was designed to create the perception of increased sperm competition risk associated with the initially preferred female and to investigate whether males are able to respond adaptively to this risk and adjust their preferences accordingly. If males are capable of such a response, we predict that focal males should spend significantly less time with the initially preferred female in stage 3 and more time with the other female.

Experiment 3: male mate choice copying

Although copying the choice of other males can increase the potential for sperm competition, male mate choice copying has been reported in another poeciliid fish, the sailfin molly (*Poecilia latipinna*; Schlupp and Ryan 1997). Consequently, to investigate such a possibility in the eastern mosquito fish, we conducted a third experiment testing the repeatability of male mate choice when focal males were able to observe a stimulus male in the vicinity of the initially nonpreferred female ($N = 14$). Here, an opaque partition during stage 2 prevented the focal male from seeing the stimulus male adjacent to the initially preferred female. If male mosquito fish copy the choice of other males, we predict that males should reduce or reverse their preferences in stage 3 and spend significantly less time with the initially preferred female in favor of the other female (Schlupp and Ryan 1997).

Statistical analyses

Two-tailed paired t -tests were used to compare the proportion of time spent associating with the initially preferred female in stages 1 and 3 for all 3 experiments. Analysis was performed using R, following arcsine square root transformation of proportional data.

RESULTS

Experiment 1: male choice in the absence of perceived sperm competition risk

We found that male eastern mosquito fish were highly consistent in their choice of female when they did not have the opportunity to witness either of the females interacting directly with other males. Specifically, as predicted, the percentage of time males spent associating with the initially preferred female did not differ between stages 1 and 3 (paired t -test: $t = 1.21$, $N = 15$, $P = 0.25$; Figure 2).

Experiment 2: male choice in the presence of perceived sperm competition risk

When focal males were given the opportunity to observe the initially preferred female interacting with a stimulus male, we found that the percentage of time spent with that female was subsequently reduced (paired t -test: $t = 3.18$, $N = 15$, $P = 0.007$; Figure 3).

Experiment 3: male mate choice copying

Focal males were consistent in the percentage of time spent with the initially preferred female despite having had the opportunity to witness another male consorting with the initially nonpreferred female (paired t -test: $t = 1.35$, $N = 14$, $P = 0.2$; Figure 4). Thus, there does not appear to be any evidence of male mate choice copying in mosquito fish.

DISCUSSION

Our study suggests that the perceived risk of sperm competition can influence male mate choice in the eastern mosquito fish. We found that males were consistent in the percentage of time spent with the initially preferred female when they did not have the opportunity to witness another male associating with her. By contrast, when focal males could see the initially preferred female associating with a stimulus male, they significantly reduced the percentage of time spent with that female and, instead, spent more time associating with the other female.

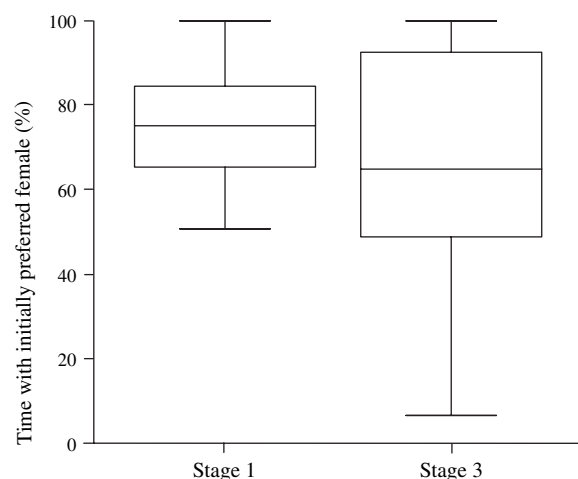


Figure 2
Time spent by males in association with the initially preferred female before (stage 1) and after (stage 3) observing both females only (Experiment 1). Box plots show twenty-fifth, fiftieth (median), and seventy-fifth percentiles, with whiskers depicting the range of the data.

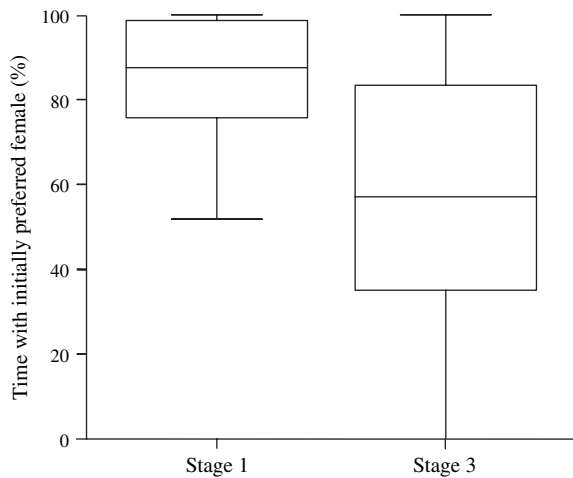


Figure 3
Time spent by males in association with the initially preferred female before (stage 1) and after (stage 3) observing the initially preferred female associating with another male (Experiment 2).

Studies investigating adaptive male responses to sperm competition risk have shown that males, in a wide range of taxa, are able to tailor their reproductive investment during mating in order to maximize their reproductive returns. A common response seen in many species of fish, for example, is to modify the quantity of sperm that is released (Candolin and Reynolds 2002; Pilastro et al. 2002; Evans et al. 2003; Zbinden et al. 2003). More recently, work on humans (Kilgallon and Simmons 2005) and crickets, *Teleogryllus oceanicus* (Simmons et al. 2007; Thomas and Simmons 2007), have shown that males may also respond to sperm competition risk by strategically adjusting the quality of their ejaculates. Our study suggests that, in addition to fine-tuning reproductive investment during mating, the perceived risks of sperm competition also can have an important bearing on precopulatory behavioral decisions through their effects on male mate choice.

Interestingly, the mere presence of another male in the vicinity of the initially preferred female was enough for male mosquito fish to adjust their association preferences. This con-

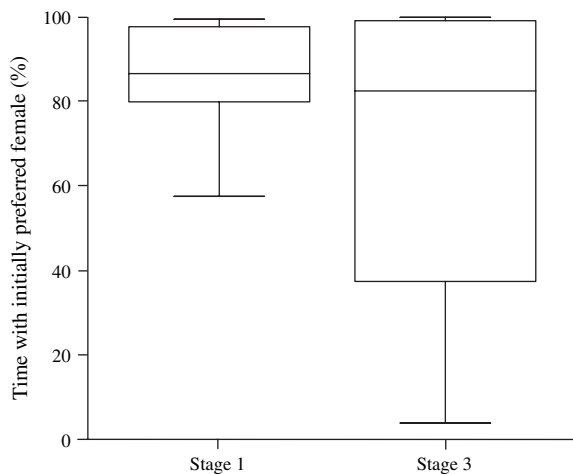


Figure 4
Time spent by males in association with the initially preferred female before (stage 1) and after (stage 3) they have had the opportunity to witness the initially unpreferred female associating with another male (Experiment 3).

trasts with the results reported in another poeciliid fish, the guppy, *Poecilia reticulata* (Dosen and Montgomerie 2004). Males, in that species, only adjusted their preferences if they were able to observe females physically mating with other males. Male guppies, however, differ from mosquito fish in their use of colorful courtship displays to solicit copulations from choosy females (reviewed in Houde 1997). Indeed, during the course of mate sampling, female guppies may routinely associate with different males without necessarily mating with them (Pitcher et al. 2003). Hence, for a guppy male, merely witnessing another male in the vicinity of a female should not necessarily diminish his value as a potential suitor. Male eastern mosquito fish, on the other hand, do not engage in any courtship activity (Bisazza 1993). Instead, copulations are obtained exclusively through rapid gonopodial thrusts directed toward females when in close proximity (Bisazza and Marin 1995). The quick and surreptitious manner in which copulations are achieved may explain why male mosquito fish are more reticent to continue consorting with a female that had previously been seen in the vicinity of another male. Nevertheless, it could be interesting to test if focal males show an even stronger aversion after seeing rival males physically interacting and/or mating with potential mates.

We found no evidence of male mate choice copying in mosquito fish. If males copied the choice of others, one might have expected them to spend significantly more time with the initially unpreferred female after seeing another male associating with her (Schlupp and Ryan 1997). This was not the case. We do not know if female mating order within a brood cycle influences male fertilization success in mosquito fish, but patterns of sperm precedence (if any) are likely to be important (Parker 1990; Evans and Magurran 2001). In some taxa, fertilization success is biased in favor of the last male to mate (Simmons and Siva-Jothy 1998). Under such a scenario, males might conceivably benefit from copying the choice of others. Otherwise, fertilizations lost through sperm competition should make mate choice copying an unappealing proposition, unless it can be used to safeguard against other kinds of reproductive costs (Schlupp and Ryan 1997).

Although reproductive costs can have important consequences for male fitness, only a handful of studies have expressly considered if, and to what extent, such costs might influence male mate choice. Male Pacific blue-eye fish, *Pseudomugil signifer*, for example, adjust their preferences in response to changes in the energetic demands of courtship (Wong and Jennions 2003), whereas male mate choice in the St Andrews web spider, *Argiope keyserlingi*, appears to be sensitive to the prospects of future mating opportunities and the risk of pre-mating sexual cannibalism (Gaskett et al. 2004). More recently, work in *Drosophila* has shown that males may also tailor their level of choosiness according to resource availability, with male stringency increasing as resources required for mating become diminished with each successive mating attempt (Byrne and Rice 2006). Such findings, considered alongside those reported in the current study, suggest that choosy males may be far more responsive to fluctuating costs than traditionally assumed. Males, it seems, have the capacity not only to be discerning about their mates but also to be every bit as sophisticated and dynamic in the exercise of their preferences as choosy females.

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