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Male mate choice and insemination success under simultaneous versus sequential choice conditions

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Theory predicts that males should be choosier when encountering potential mates simultaneously rather than sequentially because there is no opportunity cost. Consequently, when mate encounter rates vary across space and time males might benefit from plasticity in mate preferences to match prevailing social conditions, preferring high-quality mates when females are encountered frequently and showing no preferences when females are encountered rarely. Here we investigated how encounter type (i.e. simultaneous or sequential) alters male mate preferences for female size in the mosquitofish, *Gambusia holbrooki*. We found that male mosquitofish attempted to mate with a relatively large female significantly more often than a relatively small female when presented with two females simultaneously. In contrast, males showed no such preference when sequentially presented with two females. Further, males attempted more copulations with absolutely larger females irrespective of encounter type. Despite these behavioural patterns, however, neither male insemination success nor the number of sperm transferred was influenced by female size or the encounter type. Our results provide support for the prediction that male mate choice is stronger during simultaneous choice encounters, but suggest that insemination success in *G. holbrooki* is partly under female control.

Male mate choice is more likely to evolve when there is variation in female quality, males have limited resources to invest in mating and there are low costs to being choosy (Bonduriansky, 2001; Edward & Chapman, 2011). To date, most empirical studies of male mate choice have focused on identifying the targets of choice (e.g. Pack et al., 2009; Tigreros, Mowery, & Lewis, 2014) and the benefits associated with choosing particular females (e.g. LeBas, Hockham, & Ritchie, 2003; Kekalainen, Huuskonen, Tuomaala, & Kortet, 2010; Nordeide, Kekäläinen, Janhunen, & Kortet, 2013). These studies have highlighted that male mate choice can evolve in a broad range of mating systems. There is, however, far less understanding of what contributes to variation in the presence and the strength of male mate choice among populations and between species (but see Dougherty & Shuker, 2014).

A key factor in the evolution of male mate choice is the relationship between the number of receptive females (i.e. mate encounter rate) and a male's capacity to mate. Male mate choice is predicted to evolve when mate availability is high and male capacity to mate repeatedly is low (Edward & Chapman, 2011). If females are frequently encountered, it is even possible that two or more potential mates are encountered simultaneously. This makes male mate choice more likely as individuals can choose between the immediately available mates at no cost (i.e. rejection does not lower the mating rate). Consequently, even small differences in the profitability of each mating favour the evolution of choice. In contrast, during sequential encounters, choosiness lowers a male's mating rate because some females are rejected (Barry & Kokko, 2010). Simultaneous availability of mates is a general cue that mate encounter rates are likely to be high.

It is expected that when mate availability/encounter rates vary across space and time individuals should adjust their level of choosiness to the perceived mate availability (Svensson, Lehtonen, & Wong, 2010). Under low mate availability, such that mates are only sequentially encountered, individuals should take advantage of a current mating opportunity. Under high mate availability, especially if this leads to simultaneous encounters with mates, individuals should be choosier. This prediction is best studied in experiments that compare male choice in different social contexts to control for effects of variation in male 'time out' on choosiness. For example, experimental studies on fiddler crabs (*Uca* spp.) have

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shown that males do not discriminate between heterospecifics and conspecifics during sequential encounters but do during simultaneous encounters (Booksmythe, Jennions, & Backwell, 2011). Likewise, male sticklebacks, *Gasterosteus aculeatus*, and male salamanders, *Desmognathus santeetlah*, preferred to court larger females, but only when females were presented simultaneously rather than sequentially (Rowland, 1982; Verrell, 1995). This trend is widespread. Interestingly, however, when examining all available studies greater choosiness during simultaneous choice is observed for females, but not for males, indicating that males across species may respond less consistently than females to variation in encounter rate (meta-analysis: Dougherty & Shuker, 2014). Male mate choice involves decisions not only about whether to mate, but also how to allocate resources to each mating (Parker, 1998; Parker & Pizzari, 2010). For example, males can vary how much sperm they transfer depending on a female's size, condition or mating history (meta-analysis: Kelly & Jennions, 2011). Consequently it can be informative to look not only at mating behaviour but also at insemination success and the number of sperm transferred to different females. How social environments influence male allocation of sperm to females of different quality has mostly been studied in the context of sperm competition (review: Wedell, Gage, & Parker, 2002). Theory predicts that males should adjust sperm allocation in response to the risk and intensity of competition (review: Parker & Pizzari, 2010; meta-analysis: Kelly & Jennions, 2011). Less is known about how males adjust sperm allocation to other social cues. More specifically, there are few studies designed to directly compare sperm allocation under different mate encounter scenarios (for a noteworthy exception see Cornwallis & Birkhead, 2006). However, greater sperm allocation to high-quality females has been shown for males exposed to females both simultaneously (e.g. two-choice tests: Cornwallis & Birkhead, 2006) and sequentially (e.g. 'no-choice' tests: Lüpold, Manier, Ala-Honkola, Belote, & Pitnick, 2010; Rubolini et al., 2006; see also Appendix S2 of: Kelly & Jennions, 2011). These studies suggest that males can allocate sperm strategically, even during sequential mate choice.

Here we investigated how encounter type (i.e. simultaneous or sequential) affects male mate preferences for larger females in the mosquitofish, *Gambusia holbrooki*. Mosquitofish are well suited to investigating the causes of variation in male mate choice. First, they have internal fertilization and males transfer sperm to females via a modified anal fin called the gonopodium (Constanz, 1989). Males do not engage in courtship but perform coercive 'sneak' copulations in which they approach a female from behind and thrust their gonopodium towards her gonopore (Bisazza, 1993; Bisazza & Marin, 1995). This occurs repeatedly, which makes it possible to quantify male mating attempts (e.g. Booksmythe, Backwell, & Jennions, 2013). Second, female size varies considerably and is strongly correlated with fecundity (Bisazza, Marconato, & Marin, 1989; Callander, Backwell, & Jennions, 2012; Deaton, 2008). Thus, there are clear benefits to mating with larger females. Despite the likely benefits, however, male preferences for large females are not universal: some studies show a male preference for larger females (Bisazza et al., 1989; Callander et al., 2012; Mautz & Jennions, 2011), and others do not (McPeek, 1992). Furthermore, studies show that male preferences for large females can vary depending on other factors (e.g. trial type: Hoysak & Godin, 2007; mating history: Vega-Trejo, O'Dea, Jennions & Head, 2014). Third, males invest considerable effort trying to mate (attempting to copulate up to 20 times/min; Wilson, 2005) and may often make mate choice decisions when sperm stores are low (O'Dea, Jennions, & Head, 2014). Consequently, pursuing low-quality females could be costly in terms of lost opportunities to inseminate more profitable females. Finally, mosquitofish have highly dynamic social groups, forming mixed-sex shoals of varying size and sex ratio (Agrillo, Dadda, & Serena, 2008). The social environment varies widely, with the adult sex ratio and density of each sex changing throughout the breeding season (e.g. Kahn, Kokko, & Jennions, 2013). As such, males experience considerable variation in female encounter rates. Selection for plastic changes in mating behaviour given different mate encounter rates might therefore be strong.

Owing to the potential for individuals to encounter prospective mates simultaneously, studies of male mate choice in mosquitofish have only employed designs that use 'two-choice' (simultaneous) trials, measuring male association time with females presented behind dividers (e.g. Mautz & Jennions, 2011; Wong & McCarthy, 2009) and/or recording attempted sneak copulation rate in trials in which males can interact freely with two females (e.g. Hoysak & Godin, 2007; Vega-Trejo et al., 2014). To our knowledge there have been no experiments using 'no-choice' (i.e. sequential) mating trials to investigate male mate choice in *G. holbrooki*. It is therefore unknown whether males adjust their mate choice based on female encounter rate. These rate changes are exemplified at the extremes by simultaneous versus sequential encounters with receptive females.

In our experiment we independently manipulated mate encounter type and the relative size of the focal females encountered. We investigated how these two factors influenced male mate choice behaviour (number of attempted copulations) towards a focal female, insemination success (whether or not the female is inseminated) and sperm allocation (how many sperm are transferred). We predicted that (1) males will show a preference for relatively larger females and (2) if the mate encounter rate strongly influences the costs of choice then male preferences will be stronger during simultaneous than sequential trials. If the effects are weak, however, males should show a similar preference for relatively large females regardless of encounter type.

METHODS

Origin and Maintenance of Fish

Male fish were collected from two ponds (35°14′27″S, 149°5′27″E and 35°14′13″S, 149°5′55″E) in Canberra, Australia, in February 2014. The females used were first-generation laboratory-reared fish whose parents were collected from the same ponds in March 2013. Prior to the experiment all fish were housed in single-sex tanks at densities of 30–60 fish per 90 litres, and females were thus virgins. Fish were maintained at 27 °C on a 14:10 h light:dark cycle and fed *Artemia salina* nauplii and commercial fish flakes twice daily. Males were kept in the laboratory for 3–6 months prior to being used in our experiment.

Experimental Design

Each male was exposed to two females, one of which was the focal female. We independently manipulated (1) how males encountered the focal female (sequentially or simultaneously with the other female) and (2) the relative size of focal females (bigger or smaller than the other female). We then investigated the effects of these two factors and their interaction on male mate choice and sperm allocation using a 2×2 factorial design. Thus, we had four experimental treatments (sequential/relatively small female, sequential/relatively large female, simultaneous/relatively small female, simultaneous/relatively large female). Each male was assigned a unique pair of females and was only used once.

To manipulate the relative size of focal females we divided virgin females from our stock population into three size classes: small (<300 mg), medium (350–450 mg) and large (>500 mg). Female