



Revisiting and interpreting the role of female dominance in male mate choice: the importance of replication in ecology and evolution

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Abstract

In many species females prefer to mate with socially dominant males, often because it elevates their fecundity by providing greater access to material resources or lowers offspring mortality due to superior male defence. Far fewer studies have tested whether males prefer socially dominant females. Intriguingly, an earlier study showed that when the sexes freely interact, male mosquitofish (*Gambusia holbrooki*) preferentially attempt to mate with dominant females. Here we replicate this study using a slightly modified experimental design to test the generality of its finding. In addition, we conducted standard, two-choice male mate choice trials to test directly whether males prefer dominant over subordinate females. Corroborating the previous study, we found that when a male and two females freely interact, males more often attempt to mate with the dominant female. However, males did not prefer to associate with (i.e., choose) dominant females in two-choice trials where females could not interact. We discuss whether greater access to males is a benefit of female social dominance, or an epiphenomenon of other benefits of dominance.

Keywords Competition · Mate choice · Poeciliid · Replication · Reproduction · Sexual selection

Introduction

Females tend to be the choosier sex because they take longer than males to breed and return to the mating pool due to their greater parental investment (Kokko et al. 2012; Jennions and Fromhage 2017). This sex difference leads to a male-biased operational sex ratio (OSR) (Clutton–Brock and Parker 1992). Females therefore pay a lower opportunity cost than do males when rejecting a mate due to the high availability of potential mates (Jennions and Fromhage 2017; Kokko and Jennions 2008). That is, a female only faces a small delay to mating to discriminate among potential mates and select a male that elevates her fitness above that accrued from mating randomly (Achorn and Rosenthal 2020; Andersson

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and Simmons 2006). In contrast, males are generally expected not to be choosy because they elevate their fitness by mating with as many females as possible (Trivers 1972). Given a heavily male-biased OSR, a male is unlikely to locate a better-quality mate in the time it would take him to mate with any currently available female. Even so, males can be choosy in some contexts. For example, when females are abundant and vary in their quality, males can benefit by rejecting mating opportunities with lower quality partners (Edward and Chapman 2011). Indeed, when two females are simultaneously available there is no opportunity cost for a male to choose whichever female will yield a higher fitness return (Barry and Kokko 2010).

Males have been shown to discriminate between females by using morphological, visual, chemical and other cues that allow them to assess reproductive receptivity (Assis et al. 2017; Ogden et al. 2020). In some species, females have even evolved ornaments that are honest signals of her quality to potential partners. For example, a recent meta-analysis investigating the function of colourful ornaments in female birds found colouration to be positively associated with both female quality (i.e., body mass, immune response and clutch size) and a target of male mate choice (Hernández et al. 2021). Additionally, body size is often used by males as a reliable indicator of female quality: larger females tend to produce more offspring, or are better at providing parental care (Steiger 2013). When males are limited in their ability to mate by the availability of sperm or other resources required to breed, it can be adaptive for them to prefer large females (Byrne and Rice 2006; Nandy et al. 2012). Indeed, mate choice trials show that males in many species prefer larger females (Bonduriansky 2001). These studies together suggest that males can benefit from being choosy by maximising the number of offspring sired, and potentially the quality of these offspring.

If male mating preferences are based on female body condition or other indices of physical quality, then males might also prefer females based on their social rank. This is because social status within a dominance hierarchy determines how resources are shared and utilised by group members (e.g. spotted hyenas *Crocuta crocuta*; Holekamp and Strauss 2020). In principle, female dominance could therefore be an important cue for males when choosing mates, especially when dominant females are more fecund than subordinates (Hodge et al. 2008), or when dominance provides greater access to resources that benefit offspring (Wright et al. 2020). Importantly, male mate choice for dominant females could arise in any species, regardless of its effect on fecundity or maternal care, if mating with dominant females provides genetic benefits to their offspring. This will occur whenever the underlying traits that determine female dominance status are heritable, and dominance elevates a female's lifetime fitness (Edward and Chapman 2011).

A previous study reported a strong tendency for male eastern mosquitofish (*Gambusia holbrooki*) to mate with dominant rather than subordinate females when the sexes freely interacted (Chen et al. 2011). This mating pattern was not attributable to a male preference for larger females as the statistical analysis controlled for any correlation between female size and dominance rank. To our knowledge, however, no study of other species of poeciliid fishes has reported a similar link between female dominance status and male mate choice. This makes the study worthy of closer investigation.

Gambusia holbrooki are an interesting species in which to test for male mate choice: males do not provide parental care, do not court females and the cost of sperm production for future mating effort is minimal (e.g. Chung et al. 2021). In the wild the adult sex ratio can change seasonally, often with females far outnumbering males (Kahn et al. 2013). Females mate multiply and store sperm (Constantz 1984), which means that pregnant females are still potential mates. Males therefore spend most of

their time harassing females and trying to force copulations. Males do, however, show a strong mating preference for larger females, presumably because female body size is positively correlated with fecundity (Bisazza et al. 1989). Although males constantly attempt to mate, they still invest their effort strategically. For example, males can seemingly discriminate between shoals that differ in membership numbers and/or sex ratios to increase the likelihood of successful matings (Agrillo et al. 2008). Because female quality can vary due to less readily detected factors (i.e., age, reproductive status), male mosquitofish might collect information on female dominance status to make adaptive mating decisions. Females engage in aggressive interactions to establish size-based dominance hierarchies, with larger females being socially dominant (Matthews and Wong 2015). Here, we conducted a conceptual replication (i.e. a test of the hypothesis or findings of an original study to see how generalisable the results are under different conditions or across different species, see: Fidler et al. 2017; Nakagawa and Parker 2015) of the earlier study of *G. holbrooki* by Chen et al. (2011) to test its robustness. In addition, we experimentally corrected for any effect of female size and female-female interactions on male mate choice to directly test the previous interpretation that males prefer to associate with dominant females.

Methods

Animal Collection and maintenance

Adult mosquitofish were wild-caught in Canberra, Australia (35°14'30.1"S 149°06'17.0"E) during the summer of 2019–2020. Wild-caught fish were placed in covered 15 L buckets and brought back to aquarium facilities in the laboratory. Fish were kept in 90 L same-sex stock tanks (~50 individuals per tank), were maintained at 29 ± 1 °C under a 14 L:10 D cycle and were fed a mix of commercial fish flakes and brine shrimp (*Artemia salina* nauplii) *ad libitum* twice daily. Fish collection was conducted under a Scientific Licence from the Australian Capital Territory (ACT) Government, granted under Sect. 21 of the Fisheries Act 2000 (license no. FS20188). All experimental work was conducted under protocol A2021/04 of the ANU Animal Ethics Committee.

Prior to experimental trials, we isolated 150 females in individual 1 L aquaria filled with aged water, a layer of gravel and a plastic plant for cover. These females were then briefly anaesthetised in ice slurry to measure their standard body length (SL; mean \pm SD = 30.72 ± 1.72 mm) using dial callipers, before being sorted into 30 groups of five, size-matched females (< 1 mm size difference among group members). We then tagged each female in each group with a unique fluorescent elastomer tag (blue, green, pink, orange and white; NorthWest Marine Technology, Washington, USA) for individual identification. An earlier study reported a significant preference for yellow-coloured tags in female mosquitofish (Aich et al. 2020). Although this preference for yellow-coloured tags has not been reported in subsequent studies, we avoided using yellow tags in the present study. To minimise handling stress, females were tagged while their body size was measured. Tags were 1–2 mm in length and were injected subcutaneously either below the dorsal fin or along the tail close to the caudal peduncle in different combinations for individual identification. We allowed females to recover in isolation for a minimum of 2 days prior to experimental trials.

Female dominance hierarchies

Following Chen et al. (2011), we placed the five size-matched females together into 6 L aquaria filled with aged water, a layer of gravel and plastic plants for cover (so that fish could freely avoid social interactions). We lined three sides of the experimental aquaria with black plastic to minimise outside disturbance and kept the fourth side uncovered to make behavioural observations. Females were left for ~12 h to establish dominance hierarchies. After this, females were then fed a small amount of fish flake and observed for 10 min (by observer 1) to record absolute rates of aggression and to establish the dominance ranking of each individual (1–5). The social dominance trials were observations of natural interactions between the five females.

Observer 1 (always the same person) determined dominance rank by recording each time a female initiated aggressive interactions towards other group members. Both male and female mosquitofish tend to form stable dominance hierarchies (Caldwell and Caldwell 1962; Chen et al. 2011; Matthews and Wong 2015; Harrison et al. 2018). Aggressive interactions between individuals start with lateral displays or rapid approaches, then quickly escalate to biting or nipping fins and then end with the dominant individual chasing the subordinate (e.g. Harrison et al. 2021). We recorded these behaviours as aggressive interactions whenever they were initiated by one individual and directed towards another (i.e. absolute rate of aggression). Based on our observations, aggressive interactions are more intense between females of similar rank (i.e., rank 2 and 3), where more aggressive interactions were sometimes recorded, so we also noted which females won or lost chases, and which females monopolised access to food. Overall, dominant females were the most aggressive, chasing all other females, while the most subordinate females tended to avoid other fish (Fig. 1).

To assess the repeatability of our measure of dominance rank, after a 10-minute interval, a second observer (observer 2) also observed the same group of females for 10 min. Observer 2 was any one of six volunteers who was available to watch three sets of female interactions on a given day. Observer 2 quantified the same behaviours as observer 1: number of aggressive interactions initiated, the female who always chased/monopolised resources, and the female who avoided interactions. There was perfect agreement about the identity of the most dominant and most subordinate female for 24 of the 30 groups. In the remaining 6 groups, it was simply a dispute as to the rank of the first and second most dominant, or most subordinate, females. The disagreement was never such that observer 2 reversed the ranking of the females ranked 1 and 5 by observer 1. Where there was disagreement, we used the ranking of observer 1 who was more experienced. In sum, the high agreement between the observers suggests that our method was robust as even inexperienced observers could identify the most dominant and subordinate females.

Mating trials

Once dominance ranks were determined, the three females in the middle of the hierarchy were removed from the experimental tank and kept together in another tank. To ensure our ability to track individual females, we only used the most dominant and most subordinate female for the subsequent mating trial. This decision is further warranted because Chen et al. (2011) found that males did not differ in the number of mating attempts made to the three middle-ranking females.

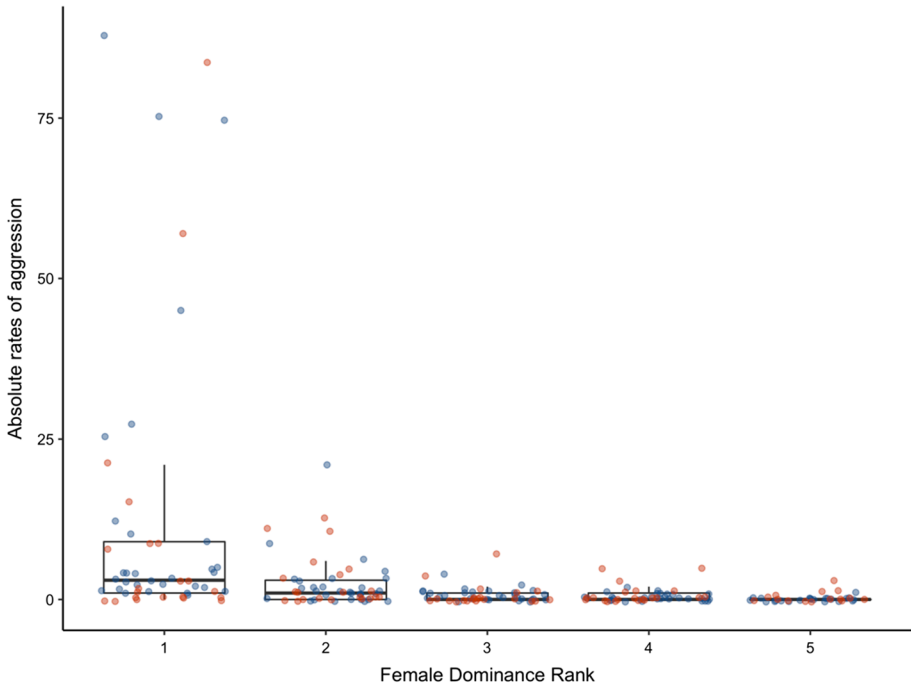


Fig. 1 Absolute rates of aggression recorded by observer 1 (red) and observer 2 (blue) during sequential 10 min observation periods of female groups. Aggressive interactions between the five females were used to rank group members from 1 to 5 (dominant – most subordinate) to identify the most dominant and most subordinate females. Boxplots show sample distribution, median, interquartile range, and minimum and maximum values

An adult male ($n=30$, SL: $\text{mean} \pm \text{SD} = 20.60 \pm 1.61$ mm) from the stock population was then placed in the experimental tank with the remaining two females (hereafter ‘dominant’ and ‘subordinate’). All three fish were given 20 min to acclimate before we observed the male’s mating behaviour for 20 min. We observed and recorded the number of mating attempts made towards each female. Male mosquitofish harass females and force copulations by swinging their gonopodium forwards and then lunging at the female before thrusting their gonopodium towards her gonopore. These mating attempts are unambiguous and easy to quantify (Bisazza and Marin 1995). Males that did not engage in any mating behaviour in the 20 min were removed and replaced with a second male ($n=13$ of the 30 sets of females) and the trial was rerun. We consider it mating behaviour if males pursued females and approached them from behind, even if they did not attempt to copulate, because mating attempts can be inhibited by aggressive interactions with females.

Mate choice tests

Immediately following the mating trial, experimental fish were moved into a two-choice experimental tank to record how long the same male spent with each female. We ran mating behaviour trials before mate choice tests so that males could gain information on the dominance status of females by observing social interactions (i.e., under natural

conditions). If males do prefer dominant females, we therefore expected his preference to carry-over to the mate choice tests. This order of testing increases the chances of detecting a male preference for dominant females. Testing the same male's mating preferences sequentially in both two-choice trials and mating behaviour trials has been done previously, but in the opposite order to our study (see Jeswiet and Godin 2011). Association time is a standard measure of attractiveness (Dougherty 2020), hence male mating preferences, in poecilids (Schlupp 2018), including *G. holbrooki* (e.g. Aich et al. 2020; Bisazza et al. 1989; Callander et al. 2012). In our mate choice tests, females could not physically interact, which removes any direct effect of female-female interactions on male access to a female. As such, we could disentangle female-female interactions and male mate choice to better test for male mating preferences for dominant females. This is the same approach used in standard tests for female mate choice: if males can fight each other, it is impossible to determine whether females prefer the winners of fights or are simply constrained to mate with winners who monopolise access to her (e.g. Shackleton et al. 2005).

The choice tank (63 × 20 × 22 cm L × W × H) was filled with water to a depth of ~ 10 cm and surrounded by opaque white plastic to minimise outside disturbance. The tank contained three compartments separated by a mesh barrier to prevent physical interactions but allowed for visual and olfactory cues. The dominant and subordinate female were randomly placed in opposite end compartments (each compartment was 7 × 20 × 22 cm L × W × H). There was no evidence for a side bias (Binomial test, $P > 0.5$). The same male from the mating trial was placed in the middle compartment (49 × 20 × 22 cm L × W × H), and, after a 5 min acclimation period, we then recorded for 15 min the amount of time he spent associating with each female. Association was defined as occurring when his whole body was < 5 cm from the barrier separating him from the female, which was indicated by a black line. We used an overhead digital camera to record the male's behaviour. We measured the male's body size at the end of the trial. The overall mean size difference between males and females was 10.21 mm (Welch's *t*-test: $t_{29} = 26.58$, $n = 30$, $p < 0.0001$).

Repeatability

At the end of mate choice trials, the two focal females were placed back into the 6 L aquaria with the other three females in their group and left for another ~ 12 h to measure the repeatability of dominance rank. To test the repeatability of our measure of the two focal females' relative dominance rank, we again ranked the females for their position in the dominance hierarchy. Over a 10 min period, observer 1 alone recorded the same aggressive behaviours as on the previous day to determine the rank of each of the five females. There was no observer 2 for these observations of dominance rank as observer 2 was only required to test the repeatability of our method of ranking females.

To assess the repeatability of our assessment of the two focal females' dominance rank before and after the mating trials we used the *rptR* package (Nakagawa and Schielzeth 2010; Stoffel et al. 2017) to run linear mixed models with individual identity (Female ID) as a predictor variable nested within group ID. Dominance rank was a binary response variable (either dominant or subordinate) to compare the relative dominance rank of focal females across the two days. Focal female dominance rank before and after mating trials was highly repeatable within groups ($R = 0.51$, 97.5% CIs 0.00, 0.70; $p = 0.001$). The relative ranking of the two females remained the same over the two days in 19 of 25 groups ($n = 5$ groups did not engage in aggressive or social behaviours on Day 2 and so dominance rank could not be established). Similar-sized female *G. holbrooki* engage in more

aggressive interactions than groups of females with more pronounced size differences (Matthews and Wong 2015), so some rank order shifts in groups of size-matched females was expected.

Statistical analysis

For the mating trials we used a generalised linear mixed model (GLMM) with a negative binomial error distribution to test whether the number of mating attempts directed towards a female was influenced by her dominance rank (dominant or subordinate). Female dominance rank and absolute female size per set (standardised across the entire dataset) were treated as fixed factors. Group ID was a random factor. Based on a decision made prior to analyses we only tested for the main effect of each factor (following the methods of the earlier study by Chen et al. (2011)).

For the mate choice tests we used a GLMM with a Gaussian error distribution to test whether the absolute amount of time a male spent with a female was influenced by her dominance rank. We chose to use absolute rather than relative time spent with a female because including group ID as a random factor accounts for the paired nature of the experiment. Female dominance rank and absolute female size (standardised across the entire dataset) were again treated as fixed factors.

Finally, we conducted a *post hoc* exploratory analysis to test for any interaction between female size and dominance rank affecting male mating behaviour. Our experimental design allowed us to control for female body size within groups (i.e., closely size-matched females), but female size across groups differed sufficiently to test whether an interaction between absolute female size and dominance affected male mating behaviour (female SL range: 27.1–34.8 mm). We therefore included an interaction term (female size x dominance rank) in our original models for both the mating trials and mate choice tests. We interpret our original models (main effects only) and exploratory models (including the interaction) separately.

All statistical analyses were conducted using R version 4.0.2 (R Development Core Team 2020). We used the package *glmmTMB* (Brooks et al. 2017) to fit GLMMs. All models included pair ID (equivalent to group ID) as a random effect to account for the paired nature of the experimental design. To find the best-fitting models, we first fit several different error distributions and link functions, then used log-likelihood ratio tests and Akaike Information Criteria (AIC) tables to identify the best-fitting model. We used the *DHARMA* (Hartig 2020) package to run diagnostics on model residuals. Model parameter estimates are presented in Tables 1 and 2. Finally, we used ANOVA type II Wald chi-square (χ^2) tests to test the significance of our model terms in the original model (and type III in the exploratory models that include the interaction term). The alpha level of significance was set at 0.05 and all tests were two-tailed.

Results

In the mating trials, on average, males directed significantly more mating attempts towards dominant than subordinate females ($\chi^2_1 = 3.84$, $p = 0.05$; Figs. 2a and 17 of 30 males directed more attempts towards the dominant female). Absolute female size did not explain the number of mating attempts a female received ($\chi^2_1 = 0.00$, $p = 0.998$; Table 1). In contrast, in the mate choice tests males showed no preference for spending more time

Table 1 Results and parameter estimates from the main effects only generalised linear mixed models for the number of mating attempts males made (mating trials) and absolute time spent with females (mate choice tests). Significant effects are indicated by an asterisk (*)

Model Parameters	Estimate	SE	z	p-value
<i>1. Mating trials – number mating attempts</i>				
Intercept	0.88	0.26	3.39	0.0007*
Dominance rank (dominant)	0.51	0.26	1.96	0.05*
Female size (standardised)	0.00	0.20	0.00	0.998
<i>2. Mate choice tests – absolute time with female</i>				
Intercept	5.35	0.09	62.20	<0.0001*
Dominance rank (dominant)	−0.05	0.12	−0.37	0.71
Female size (standardised)	0.13	0.06	2.17	0.0297*

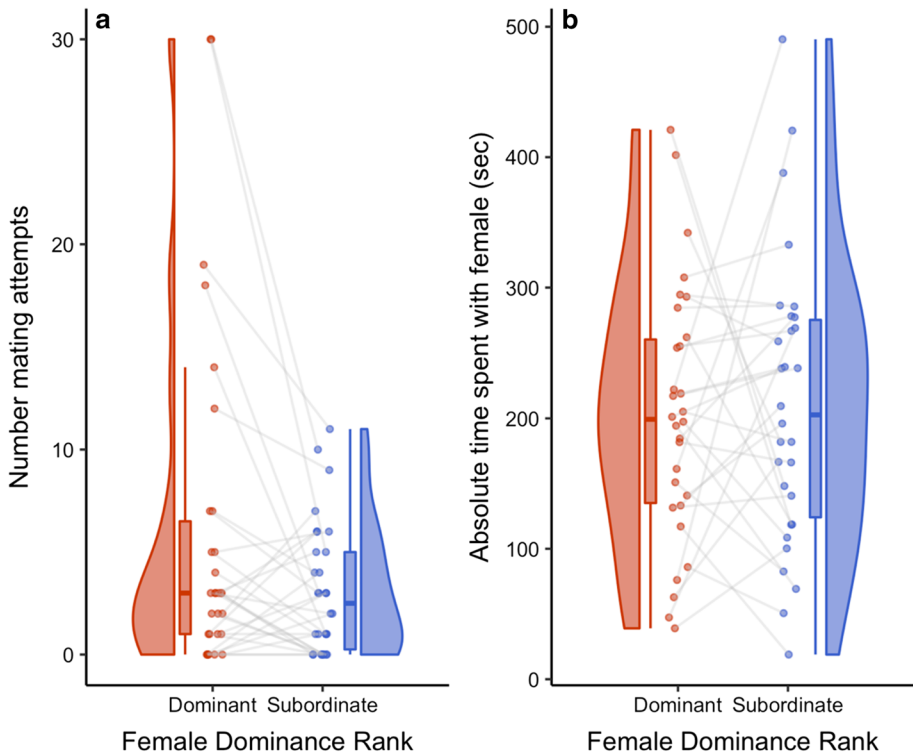


Fig. 2 Male mating bias towards dominant (red) or subordinate (blue) females in **a** mating trials and **b** mate choice tests. In mating trials, fish interacted freely and male preference was recorded as the number of mating attempts. In mate choice tests the same male was given 15 min to associate with either of two females (females behind a mesh barrier on opposite sides of choice tank). Density plots show the distribution of the data, and boxplots represent the median, interquartile range, and minimum and maximum values (excluding outliers). Grey lines link an individual male’s mating behaviour

Table 2 Exploratory results and parameter estimates from generalised linear mixed models. Here we include interactions between dominance rank and female size across groups on the number of mating attempts males made (mating trials) and absolute time spent with females (mate choice tests). Significant effects are indicated by an asterisk (*)

Model Parameters	Estimate	SE	z	p-value
<i>1. Mating trials – number mating attempts</i>				
Intercept	0.81	0.25	3.18	0.001*
Dominance rank (dominant)	0.51	0.23	2.18	0.03*
Female size (standardised)	0.31	0.26	1.21	0.23
Dominance rank x Female size	-0.51	0.25	-2.07	0.04*
<i>2. Mate choice tests – absolute time with female</i>				
Intercept	5.35	0.09	61.19	<0.0001*
Dominance rank (dominant)	-0.04	0.13	-0.35	0.73
Female size (standardised)	0.13	0.09	1.49	0.14
Dominance rank x Female size	0.00	0.12	-0.03	0.98

associating with the dominant female ($\chi^2_1=0.14$, $p=0.71$; Fig. 2b; only 9 of 30 males spent more than 50% of their total association time with the dominant female). Males did, however, spend significantly more time associating with females in pairs where both females were larger ($\chi^2_1=4.73$, $p=0.0297$; Table 1), hence males were choosy regarding absolute female size. It should be noted that female body size did not differ significantly between the dominant and subordinate female within each group (paired t -test: $t_{29}=1.16$, $n=30$ pairs, $p=0.255$). Of the 17 males that preferred the dominant female in the mating trials, six males retained their preference for her, six spent more of their time with the subordinate female, and five had no obvious preference in the mate choice tests.

Exploratory analysis

We decided *post hoc* to conduct an additional, exploratory analysis to test whether male mate choice preferences were affected by absolute female size. In this analysis, the strength of the interaction between absolute female size and dominance rank differed for the mating trials and mate choice tests (Table 2). In the mating trials, the difference between the number of mating attempts directed towards dominant and subordinate females decreased as female size increased (dominance rank x female size: $\chi^2_1=4.27$, $p=0.04$; Fig. 3a). In contrast, in the mate choice tests there was no significant interaction between dominance rank and absolute female size affecting the time males spent with a female (dominance rank x female size: $\chi^2_1=0.001$, $p=0.98$; Fig. 3b).

Discussion

Male *Gambusia holbrooki* directed marginally significantly more mating attempts towards dominant than subordinate females. Our previous work on *G. holbrooki* shows that the number of mating attempts males make is usually closely associated with the time spent in association with a female (Callander et al. 2012). Although we only gave males access to the most dominant and most subordinate of five females, our findings for the mating

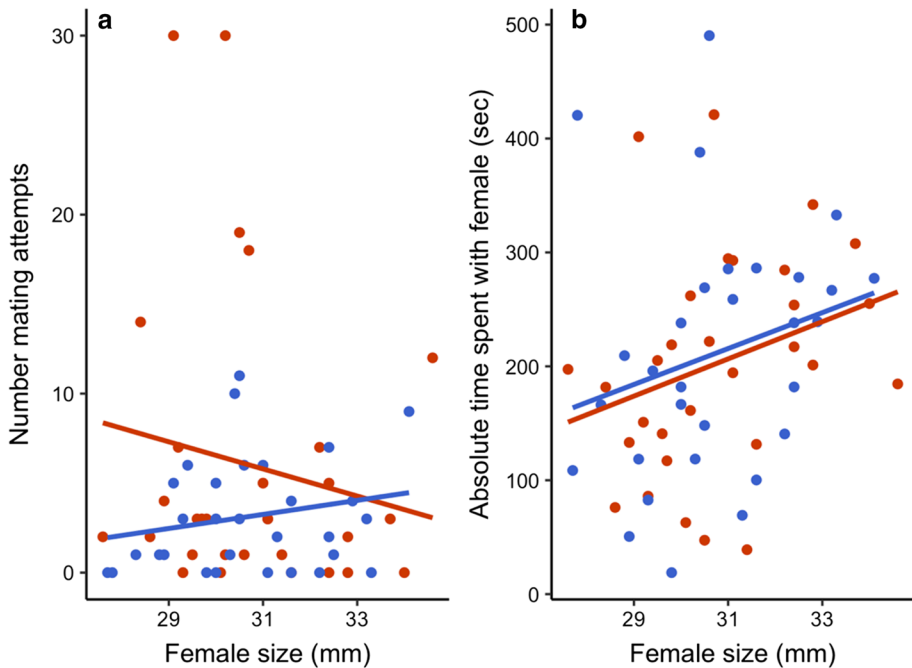


Fig. 3 Interactions between absolute female size and measures of male mating activity directed towards dominant (red) and subordinate (blue) females. In **a** mating trials, dominant females received fewer mating attempts than subordinate females as the absolute size of the females increased. In **b** mate choice tests, males showed no preference for females based on dominance rank, but spent more time associating with pairs of larger females

trials somewhat support those of Chen et al. (2011) who allowed the male to interact with all five females. However, once we controlled for female-female interactions in the mate choice tests by preventing females from physically interacting with each other, males no longer spent more time with dominant females. It is possible that the lack of male preference in mate choice tests could be due to sperm depletion after the mating behaviour trials reducing the males' sexual interest. However, we think this is highly unlikely given that in *G. holbrooki*: (a) males spend a large proportion of their time harassing females, (b) males maintain a high rate of copulation attempts even under thermal stress (Wright, 2005), or when the tip of their gonopodium is experimentally removed (Chung et al. 2019), and (c) mating attempts are mostly unsuccessful (i.e., the male's gonopodium does not contact the female's gonopore so there is no transfer of sperm bundles).

In combination, the results of the mating trials and mate choice tests suggest that dominant females can influence male mating behaviour through female-female interactions. The authors of the original study (Chen et al. 2011) suggest that this finding could be because dominant females monopolise mating opportunities with males by aggressively chasing away subordinates. Indeed, in some species, females compete with each other to obtain mates when suitable partners are limited (Forsgren et al. 2004), such as monogamous species (Campbell 2004), or species where males provide parental care or other material resources that elevate female fitness (Stockley and Bro-Jørgensen 2011). However, in *G. holbrooki* there is sexual conflict over mating whereby females try to

avoid male copulation attempts (Dadda et al. 2005) making it unlikely that females compete for mates, especially given that males provide neither material resources nor parental care. We instead suggest that dominant females are not trying to monopolise mating interactions *per se*, but rather the benefits to females from chasing away subordinate females are greater than the associated costs of attracting increased male harassment. For example, an experimental study of *Gambusia affinis* found that although female-biased sex ratios reduced the amount of male harassment each female received, female growth rates and fecundity were negatively associated with increased female density (Smith and Sargent 2006). That is, females imposed greater costs on each other than did males.

An alternative explanation for our results is that male *G. holbrooki* prefer dominant females but rely on cues produced during dominance interactions and do not retain this information (i.e., they cannot identify the dominant female later). In some species, females possess ornaments that have the dual function of signalling both competitive ability to other females and her quality as a mate. For example, in the sex-role-reversed pipefish (*Syngnathus typhle*) colourful ornaments are honest signals of female fecundity (Berglund et al. 1997). In experimental contests where male pipefish could observe female-female interactions, females displayed their ornament more strongly to other females than they did to the observing male (Berglund and Rosenqvist 2000). However, male pipefish can seemingly still use prior information on which females displayed more strongly during contests to choose the more dominant of two females (Berglund and Rosenqvist 2000). Even in the absence of ornaments in *G. holbrooki*, it remains plausible that males could detect dominant females using other cues, such as hormones or other chemicals, that could be detected by males during mate choice tests. For example, dominant female zebrafish (*Danio rerio*) release waterborne pheromones that suppress the reproduction of subordinates (Gerlach 2006), and these could potentially also act as cues of dominance status to males in eastern mosquitofish.

Female body size is an obvious cue that males can use to make mating decisions when females vary in quality. For many species, including poeciliids like *G. holbrooki*, female fecundity is positively correlated with body size (reviewed in Schlupp 2018). Where dominance rank is also determined by female body size, males might maximise their reproductive success by choosing larger, hence more dominant, females. But would this reflect a preference for size or for dominance? In the present study we observed a marginally significant effect of absolute female body size on male mating behaviour in both the mating trials and mate choice tests. Unlike the finding in the original study (Chen et al. 2011), in our mating trials there was a significant interaction (albeit a p -value of 0.04) between absolute female size and dominance rank that affected male mating attempts. In the mating trials, the difference in the number of mating attempts directed towards dominant and subordinate females decreased as female size increased. In the mate choice tests, males spent more time associating with pairs of larger females, but they did not spend relatively more time with the subordinate (or dominant) female (i.e., no interaction). This is consistent with previous work on male mate choice in *G. holbrooki* where males are more attentive towards larger females (Bisazza et al. 1989; Hoysak and Godin 2007). Our results are also consistent with female size rather than dominance status driving male mate choice, although we cannot exclude the possibility that males were using body size as a cue for female dominance status in the absence of female-female interactions during mate choice tests. It should be noted that we did not test for an effect on male choice of relative female body size within the pair of test females: our close size matching of females in each group made it implausible that we

would detect such an effect. This within-group size matching was required to experimentally minimise any correlation between female dominance rank and body size so that we could directly test for any effect of dominance rank on male choice.

Female-female competition is not always for mates and often occurs when resources are limited. Aggressive females tend to acquire more resources that can then be invested into reproduction (e.g. greater fecundity, (Cain and Ketterson 2012); larger offspring, (Wu et al. 2019)). Although dominance status can determine access to resources, it can be energetically demanding to be more aggressive (Seebacher et al. 2013). For female *G. holbrooki* the costs of female-female social interactions are seemingly far greater than those imposed by male harassment (Smith and Sargent 2006). Social interactions between females require elevated levels of aggression that disrupt feeding and temporarily prevent access to food resources, all of which determine individual growth rates and future investment into reproduction (e.g. Borg et al. 2012). As such, dominant females need to balance the energetic cost of maintaining their status through increased aggression against the benefit of monopolising food resources. While our findings show that dominant female mosquitofish receive more male mating harassment, it seems likely that this is an epiphenomenon of being more aggressive, and being even more so when a male is present. For example, in guppies (*Poecilia reticulata*), females direct more aggression towards each other when a sexually-harassing male is present than when in female-only shoals (Darden and Watts 2012). Future work is needed to better understand what benefits, if any, males gain from disproportionately mating with dominant females in such cases.

Finally, replication is fundamental to scientific progress, but very few published studies are replicated. When they are, the results are often discordant with those originally reported (Aarts et al. 2015). This is problematic and has led to widespread concerns in many fields (e.g. the so-called ‘replication crisis’ in psychology). The problem of low replication also applies to ecology and evolutionary biology where studies, even those with novel or unexpected results, are rarely replicated (Jennions and Møller 2002; Kelly 2019). Here, our study explicitly replicates an earlier study by Chen et al. (2011) which produced a seemingly unusual result, namely that males preferentially mated with dominant females in a species where there is no obvious link between female dominance and male fitness. This made the study an obvious candidate for replication. We successfully reproduced this earlier finding, which is a reassuring corroboration of its validity. However, by extending the original study, we also challenged the interpretation. When we controlled for the effect of female-female interactions by using conventional mate choice tests we found that males do not prefer to associate with dominant females. Our findings highlight the importance of conducting conceptual replication in the field of ecology and evolution.

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Author contributions LMH and MDJ conceived the idea for this experiment. LMH collected and analysed data and wrote the first draft of the manuscript. MDJ provided statistical advice and critical revisions. All authors reviewed the manuscript submitted for publication.

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Data and code availability All data and code used for statistical analysis is provided as Supplementary Material.

Declarations

Conflict of interest The authors declare no conflict of interest.

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