

Research



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Animal behaviour

Does the winner–loser effect determine male mating success?

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Winning or losing a fight can have lasting effects on competitors. Controlling for inherent fighting ability and other factors, a history of winning often makes individuals more likely to win future contests, while the opposite is true for losers (the ‘winner–loser effect’). But does the winner–loser effect also influence a male’s mating success? We experimentally staged contests between male mosquitofish (*Gambusia holbrooki*) such that focal males either won or lost three successive encounters with stimulus males. We then placed a size-matched (to control for inherent fighting ability) winner and loser with a female and monitored their behaviour ($n = 63$ trios). Winners spent significantly more time associating with the female. Winners did not make more copulation attempts, nor have a greater number of successful attempts. There was, however, a significant effect of male size on the number of successful copulation attempts: success decreased with male size for losers, but size had no effect on the success rate of winners.

1. Background

There is an intriguing phenomenon whereby the outcome of previous contests can have long-lasting effects on contestants when they encounter new rivals. Carry-over effects from prior contests are known as ‘winner–loser effects’: winners behave more aggressively in future contests and tend to win, while losers become submissive, are less likely to escalate contests and tend to lose [1]. The winner–loser effect is particularly apparent in male–male contests, where males often try to determine their opponent’s resource holding potential (RHP) [2], as the relative RHP of opponents tends to determine the outcome of a contest [3].

Variation in RHP arises from a combination of factors (e.g. body size, weaponry), which can also act as cues or signals to assess rivals. In male–male contests, larger males or those with the largest weapons are perceived to have greater RHP and usually win contests against smaller opponents [2,3]. In addition, differences in the value placed on a resource can affect the contestants’ motivation to fight for it, which can then influence the outcome [3]. Controlling for differences in RHP, the mere act of winning can increase a male’s future fight success. The winner–loser effect seems to be related to a process whereby past contest successes elevate a male’s assessment of his own RHP [1]. In addition, in some species, females prefer males who are socially dominant (e.g. [4]). Consequently, even after controlling for RHP, a winner–loser effect might cause winners to act in a socially dominant manner that then increases their sexual attractiveness.

To our knowledge, no study has yet shown that a winner–loser effect in males elevates the mating success of winners when males compete against each other. Following a fight, losing male *Drosophila melanogaster* flies lost motivation to mate compared to winning or naive males, but winning and losing males were not competing for the same female [5]. In *Acheta domesticus* crickets the winners of natural fights were more attractive to females, but a

subsequent test that eliminated the winner–loser effect showed that this result was simply due to the same traits elevating both RHP and attractiveness [6]. Here we explore the idea that the winner–loser effect could affect male mating success. We first experimentally manipulate how often males won or lost a series of fights. We then tested whether size-matched males with a recent history of winning mated more often than losers when competing for the same female.

We examined the potential effects of a male's previous fighting experiences in the Eastern mosquitofish (*Gambusia holbrooki*). This species is a good model organism to test whether winner–loser effects influence mating opportunities because males interact aggressively to establish dominance hierarchies [7]. Furthermore, males rarely perform courtship displays, and instead attempt to forcefully 'sneak' copulate with females [8,9]. Consequently, female cooperation is not essential for males to mate successfully. Finally, males spend a large proportion of their time harassing females and attempting to copulate, which makes it possible to estimate male mating success using behavioural data.

2. Material and methods

All test fish were the offspring of wild-caught fish captured in Canberra and maintained in single-sex stock populations under a 14 L: 10 D cycle at $27 \pm 1^\circ\text{C}$. Mature males were briefly anaesthetized in an ice slurry to photograph them and measure their body size (standard length, SL) using *ImageJ* [10]. They were then individually held in 1.2 l tanks for 10 weeks prior to contests so that they had no recent fighting experience.

In stage 1, winners and losers were created by staging fighting contests between size-mismatched males (focal and stimulus) such that each focal male either won or lost three successive encounters [11]. Focal males were randomly assigned either a winning or losing experience. For winners, stimulus males were 3.58 mm smaller (mean SL); and for losers, stimulus males were 3.36 mm larger (mean SL). Winner and loser contest trials were conducted simultaneously for a pair of size-matched focal males (less than 0.1 mm difference in SL, such that winners did not differ in mean body size from losers (paired *t*-test: $t = 0.09$, $p = 0.93$; $N = 63$). Prior to stage 1, one of the males (picked at random) was marked with a subcutaneous elastomer tag to distinguish between the winner and loser during stage 2. Stage 1 contests took place in $5 \times 19 \times 19$ cm aquaria. Males were given 10 min to acclimate (kept behind mesh barriers with only visual contact) before the barriers were removed and the males could freely interact for a further 10 min. Focal males participated in three contests, each time with a new rival. There was a 10 min interval between successive contests. During the contests males engaged in aggressive displays, and nipped and chased each other.

Immediately following stage 1, the size-matched winner and loser were allowed to compete against one another for the same female (stage 2). The female was an adult virgin randomly taken from the stock population and placed in the mating contest aquarium (6.5 l) 10 min before the males. The focal male pair was then placed in the tank with the female and observed for 10 min. We recorded the number of copulation attempts each male made (his gonopodium rotated forward as he approached the female), the number of successful copulation attempts (his gonopodium contacted the female's gonopore) and the time (s) spent close to the female (approx. less than 3 cm). When a competitive mating trial ended the female was anaesthetized in an ice slurry and photographed to measure body size using *ImageJ* [10].

Data were analysed using separate generalized linear mixed effects models for each response variable: namely, time spent associating with the female (Gaussian distribution), number of copulation attempts (Poisson distribution corrected for overdispersion using an observation level random effect) and number of successful attempts (Poisson distribution). Male treatment (winner/loser) was treated as a fixed effect and competitive mating trial ID as a random effect. *P*-values were obtained from Type III Wald's χ^2 tests. We initially ran models with only male treatment as a fixed effect. We then ran *post hoc*, exploratory models that included standardized (mean = 0, s.d. = 1) male and female size as fixed covariates; and included their interaction with male treatment. There was a significant interaction for male copulation success, so we ran separate Pearson's correlation tests for winners and losers for the relationship between male size and copulation success. All statistical analyses were conducted using R v. 3.4.1 [12].

3. Results

Parameter estimates are presented for models either excluding (electronic supplementary material, tables S1–S3) or including male and female size (electronic supplementary material, tables S4–S6).

In the planned models, winners spent significantly more time than losers associating with females ($\chi^2 = 5.34$, $p = 0.02$) (figure 1a). However, winners did not make significantly more copulation attempts than losers ($\chi^2 = 0.99$, $p = 0.32$) (figure 1b); nor did winners make significantly more successful copulation attempts than losers ($\chi^2 = 1.56$, $p = 0.21$) (figure 1c).

In the *post hoc* models, neither male nor female size influenced the relative amount of time winners versus losers spent associating with females (treatment \times female size: $\chi^2 = 2.08$, $p = 0.15$; treatment \times male size: $\chi^2 = 0.17$, $p = 0.68$), nor the relative number of copulation attempts (treatment \times female size: $\chi^2 = 0.01$, $p = 0.91$; treatment \times male size: $\chi^2 = 1.26$, $p = 0.26$). There was, however, a significant interaction between male size and being a winner or loser affecting the number of successful copulations ($\chi^2 = 6.48$, $p = 0.01$). Copulation success decreased with male size for losers, but remained unchanged with size for winners (statistics in figure 2). Finally, larger females were the recipients of a significantly greater number of successful copulations from both winners and losers (female size: $\chi^2 = 5.22$, $p = 0.02$).

4. Discussion

Winner males spent more time associating with females, either through more persistent harassment or by chasing away the loser. In mosquitofish, the winner effect might increase the frequency of male pursuit of females if the experience of winning boosts androgen production and male harassment is correlated with higher androgen levels, as is courtship behaviour in other fishes (e.g. [13]). Males that lose aggressive contests often incur greater costs than those that win them (e.g. energetic costs, physical damage) [1], so losers might avoid further interactions immediately following contests while recovering (loser effect). This might also explain why losers spent less time associating with the female.

Surprisingly, despite spending more time near the female, winners did not attempt more copulations, or have a greater

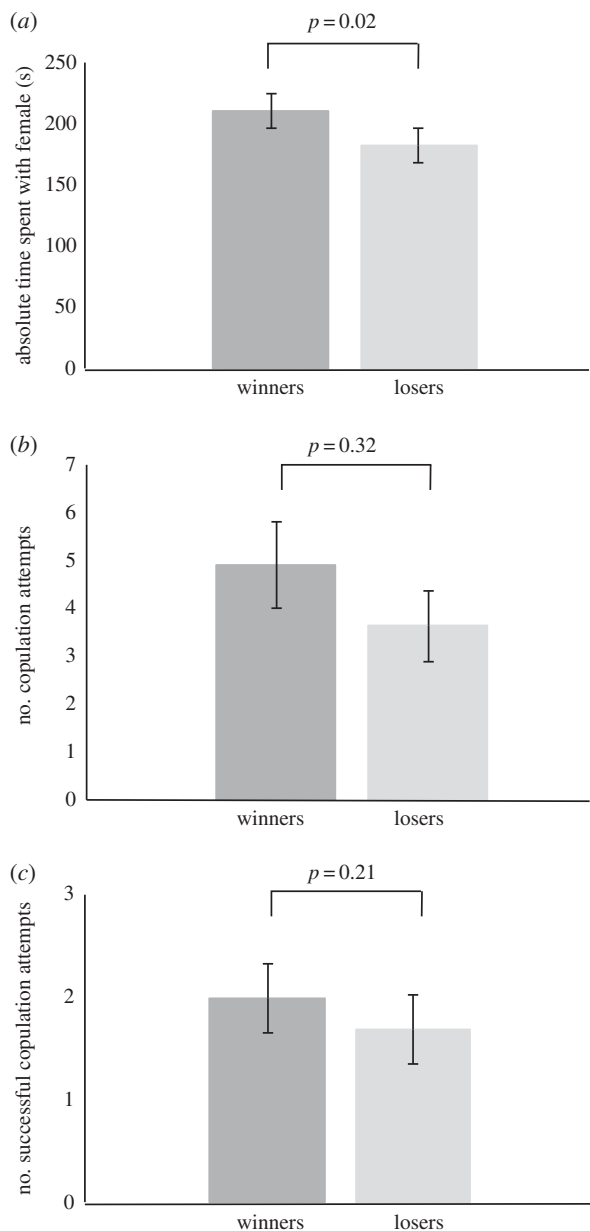


Figure 1. Behaviour of winners and losers. (a) Time spent associating with female, (b) number of copulation attempts and (c) number of successful copulations. Mean \pm s.e.

number of successful copulation attempts than losers. One explanation for this is that female mosquitofish might prefer to associate with losers to reduce the costs of sexual harassment (e.g. reduced foraging time, physical damage) [14]. Changes in sexual behaviour following fights could affect a male's attractiveness to females, as occurs in many vertebrates [15]. For example, female *Poecilia mexicana* preferred to associate with losing males after watching male–male contests, and females who associate with losers are less likely to be harassed [16]. In mosquitofish, females actively evade males [14], which makes it difficult for winners to convert their increased access to females into copulations. This might explain the similar number of copulation attempts made by winners and losers. Our study allowed males and females to freely interact during competitive mating trials to reflect a natural setting. However, controlling for female preferences might reveal a significant difference in the propensity to attempt copulations between winners and losers

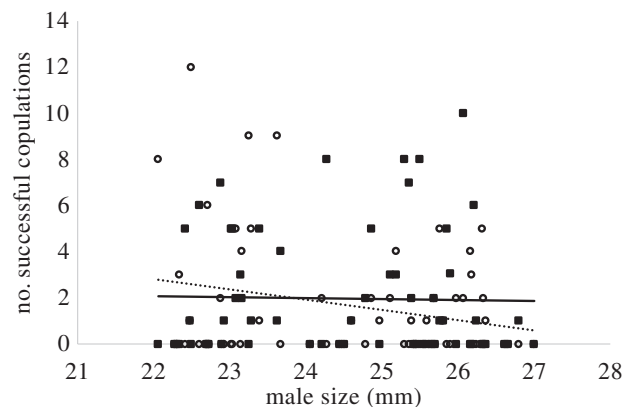


Figure 2. The effect of male body size on the number of successful copulations by winners (black, squares) and losers (white, circles). Separate regression lines are drawn for winners (solid) and losers (dashed) ($N = 63, 63$). Loser success correlated with body size ($r = -0.24$; $p = 0.06$), but that of winners did not ($r = -0.02$; $p = 0.89$).

(e.g. [5]). It is, however, generally true that increased time with a female predicts mating success in *G. holbrooki* because it elevates the number of copulation attempts that a male can make (e.g. [17]). As such, we cannot exclude the possibility that a prolonged period of behavioural observations might reveal a mating advantage for winners.

Finally, there was a significant interaction between a male's past contest experience and his body size that affected the number of successful copulation attempts he made. The benefits of being a winner did not improve as body size increased, but losers were less successful as they got larger. This pattern might be explained by the fact that small males tend to lose naturally occurring contests, while large males tend to win. As such, smaller males might be predisposed to recover more rapidly (e.g. physiologically) from a loss than larger males and so quickly resume normal mating activity.

5. Conclusion

Winner–loser effects have consequences for male success in contests, but here we show that a male's past success also affects his ability to gain access to females. Depending on female responses, males might convert greater access to females into higher mating success. Future studies should, therefore, investigate whether female preferences modulate the role of winner–loser effects in determining male mating success.

Ethics. Work was undertaken under A.N.U. Animal Ethics permit A2105/07.

Data accessibility. The dataset supporting this article has been uploaded as electronic supplementary material.

Authors' contributions. L.M.H. carried out data collection. All authors participated equally in the study design, data analysis and drafting the manuscript. All authors gave final approval for publication and agree to be held accountable for published content.

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