

Experimental evidence that winning or losing a fight does not affect sperm quality in a field cricket

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Abstract

Fighting is a powerful social experience that can affect male reproductive behavior, including ejaculatory strategies. Whereas winners may monopolize females, losers may instead perceive high sperm competition and limited future mating opportunities, and accordingly enhance ejaculate quality to maximize their reproductive success. In male field crickets *Gryllus bimaculatus* that fight aggressively for control of breeding territories, winners are known to possess sperm of lower quality (viability) compared to losers, but it remains unclear whether this is due to short-term fighting consequences. To test if the fighting experience per se (winning or losing) affects male adjustment of sperm viability, we subjected males to winning and losing experiences by staging fights against size-matched rivals of known fighting ability. These rivals were males that previously won or lost a fight and, due to “winner-loser effects” kept winning or losing subsequent contests. We sampled sperm prior and after the fight and twice in control males with no fighting experience and found no differences in sperm viability across measures. We conclude that males do not tailor their ejaculate quality following a single fight, or based on its outcome. Intrinsic differences in other attributes between winners and loser phenotypes may explain differences in sperm quality previously described in this system.

KEYWORDS

aggression, ejaculate, fertilization, field crickets, intra-sexual selection, winner-loser effects, trade-offs

1 | INTRODUCTION

Fighting represents a powerful social experience that can influence an individual's behavior with important repercussions for its survival and reproduction (Qvarnström & Forsgren, 1998). Little is known, however, about how fighting and its outcome affect male post-mating strategies. Perception of sperm competition risk and future mating opportunities may differ between males winning or losing a fight, triggering rapid adjustments of ejaculate characteristics to

maximize fertilization success. Sperm competition theory predicts that males should increase their allocation to mating, and produce ejaculates of greater sperm numbers and/or quality when competing against another male for fertilization of eggs, or when occupying a disadvantaged mating (e.g., sneaking) role (Parker, 1990a; Parker, 1990b; Parker & Pizzari, 2010). Whereas by holding a resource (mates or territory), contest winners generally monopolize females and prevent rivals from gaining fertilizations, losers may instead perceive high sperm competition risks and limited future

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mating opportunities (Parker, Ball, Stockley, & Gage, 1997). This may explain the drop in sperm number and/or quality of contest winners reported for the Arctic charr (Rudolfson Figenschou, Folstad, Tveiten, & Figenschou, 2006) and the domestic fowl (Pizzari, Cornwallis, & Froman, 2007), and the increase of sperm numbers in losers described in the broad-horned flour beetle (Okada, Yamane, & Miyatake, 2010). A strenuous fight may also induce physiological consequences, such as increasing cell oxidative stress levels known to be deleterious to sperm (Tremellen, 2008), with more aggressive males, generally winners, investing less in antioxidants and thus producing lower quality ejaculates (Mora et al., 2017).

Disentangling short-term effects of experiencing a fight from long-lasting male quality effects (Sheldon, 1994) on ejaculates is not trivial. Dominant contest winners with higher fighting abilities might also possess higher quality ejaculates owing to an overall superior body condition (Koyama & Kamimura, 2000; Thomas & Simmons, 2009). On the other hand, increased investment in traits for winning contests (e.g., body size, weapons) may be coupled with a reduction in allocation to ejaculate (Simmons, Lüpold, & Fitzpatrick, 2017) leading to negative correlations between traits enhancing fighting and fertilization success (Froman, Pizzari, Feltmann, Castillo-Juarez, & Birkhead, 2002).

We conducted an experiment designed to understand whether males tailor their ejaculates following the outcome of a fighting experience per se (winning or losing). We used the field cricket *Gryllus bimaculatus*, which engages in same-sex aggressive contests (Adamo & Hoy, 1995) over breeding territories. Field crickets are known to respond to the level of sperm competition risk by modulating sperm investment (as for *Gryllus veletis* (Schaus & Sakaluk, 2001), *Grylloedes supplicans* (Gage & Barnard, 1996), *Teleogryllus oceanicus* (Simmons, Denholm, Jackson, Levy, & Madon, 2007; Thomas & Simmons, 2007), and to some extent *Gryllus sigillatus* (Schaus & Sakaluk, 2001) and *Acheta domesticus* (Gage & Barnard, 1996)). Studies addressing how male dominance affects ejaculate characteristics such as sperm viability (the proportion of live cells in the ejaculation), a crucial trait for obtaining fertilizations (García-González & Simmons, 2005), however, show contrasting results. In the Australian *Teleogryllus oceanicus*, dominant males (winners of repeated fights) produce sperm of higher quality compared to subordinate (Thomas & Simmons, 2009). As sperm quality was measured prior the fights, these findings suggest that males possessing higher pre-mating abilities might also possess higher post-mating abilities. On the contrary, in *Gryllus bimaculatus*, contest winners (of a single fight) produce sperm of lower quality compared to losers (Tuni, Perdígón Ferreira, Fritz, Muñoz Meneses, & Gasparini, 2016). Since in the latter, sperm quality was measured following the fight, it remains unclear whether these differences stem from the outcome of the fighting experience or from pre-existing differences in individual characteristics between winner and loser phenotypes. Here, we experimentally assigned males an experience of winning or losing by staging contests with rivals of known fighting ability (Rutte, Taborsky, & Brinkhof, 2006). Knowledge on rivals' fighting ability was obtained due to "winner-loser effects"

(Khazraie & Campan, 1999), where winners are more likely to win subsequent fights and losers to be defeated (Chase, Bartolomeo, & Dugatkin, 1994; Rutte et al., 2006). In the field cricket *Gryllus bimaculatus*, the effect of a contest on male subsequent fighting behavior is reported to last for up to 6 hr after the fight (Khazraie & Campan, 1999). Hence, we staged fights between size-matched focal males and rivals which had just lost or won a previous fight, using rival winners as stimuli to impose losing, and rival losers to impose winning. Sperm viability was assessed in focal males prior to and after a fight, and twice in control males with no fighting experience. By taking advantage of an experimental design that imposes winning and losing to males similar in their body mass and by assessing sperm viability prior and after a fight, we are able to exclude any effect of pre-existing differences in male body condition and sperm quality. We could therefore target the experience of fighting per se as the sole causal effect of any observed differences in sperm viability. If males adjust their ejaculates following a fight, we expect winners to reduce and/or losers increase sperm viability due to perceived limited and enhanced sperm competition, respectively.

2 | METHODS

Crickets were collected in Italy (Tuscany) during summer 2015. They were reared at 26°C, 65% humidity, and 14:10 hr light: dark cycle in several large plastic tanks (35 × 27 × 20 cm³) containing approximately 30 individuals. These were provided with carton shelters and with ad libitum water (vials plugged with cotton), and food (dry cat food (Purina®) and fresh apple slices). Shortly before reaching adulthood, males were moved individually to plastic containers (10 × 10 × 9 cm³). All males were used 14–18 days post-adult eclosion. On the day before the experiment, spermatophores were removed from males using soft forceps and discarded to standardize subsequent spermatophore age. All males were marked with a dot of acrylic paint for individual recognition and weighed to the nearest 0.01 g using a digital scale (KERN PKT, KERN & SOHN GmbH, Germany) to match males of similar body mass into groups of individuals consisting of fighters (rival and focal) and control.

2.1 | Pre-fight sperm assay

Prior to fights, we collected a spermatophore (number 1—pre-fight) from each focal male (fighters and controls) to estimate sperm viability using established protocols (Tuni et al., 2016). Spermatophores were placed into 200 µl of Beadle saline for 10 min to release their contents once the evacuating tube was cut with scissors. The diluted ejaculate (100 µl) was stained using the LIVE/DEAD® sperm viability kit (Invitrogen, Molecular Probes Inc). Live (stained green) and dead (stained red) cells were viewed under a fluorescence microscope (Olympus BX61; Olympus), and a total number of 500 cells were counted per sample.

2.2 | Fights

Two males were trained to become either rival winners or losers by engaging in a fight; they were placed into a transparent plastic container ($15 \times 15 \times 10 \text{ cm}^3$) and allowed to freely interact for a total of 2–3 min, a laps of time that generally ensures a detectable fight outcome (Tuni et al., 2016). Fighting in crickets consists of a quick escalated sequence of behaviors (e.g., antennae fencing, mandible spreading, singing, wrestling, and biting) and ends with the winner chasing a fleeing loser (Adamo & Hoy, 1995). We scored winner and losers by identifying which of the two males chased and which fled, and assigned each rival male to one of our focal males to impose losing by fighting against the recent winner, and *vice versa* to impose winning by fighting against the recent loser. Fights between rival and focal males were staged using the same procedure, and winners and losers scored. After the fight, all males were returned to their housing containers.

2.3 | Post-fight sperm assay

On the day following the fights, spermatophores were collected from focal losers, winners and control males (those that did not experience a fight) and tested for sperm viability following the procedures described above.

2.4 | Statistical analysis

We tested a total of 82 males, 21 from control (C) and 61 from fighting treatments (30 losers-L and 31 winners-W). We used R v 3.3 (R Core Team, 2016) with the R package “lme4” to conduct a mixed-effect model analyses on sperm viability. In our model, we included male treatment (C, L, W), spermatophore order (pre- and post-fight) and their interaction as fixed effects, and male identity as a random effect to account for repeated measures (two spermatophores were collected for each male). Sperm viability was analyzed assuming a binomial distribution, and we included an observation-level factor to account for overdispersion. *p*-values for the fixed effects were obtained using the “ANOVA” function in the package “car”. ANOVA was used to test for differences in male body mass between treatment groups.

3 | RESULTS

Three males, one for each treatment, produced empty pre-fight spermatophores leaving us with a sample of 79 spermatophores. Among the males assigned to fights, 6.9% (2/29) did not lose and 6.7% (2/30) did not win. These were excluded from further sperm assays. Fourteen males did not produce a post-fight spermatophore (2 C, 6 L, and 6 W), and 3 produced empty spermatophores (1 L and 2 W). Overall, we were able to analyze the contents of two spermatophores (pre-fight and post-fight) from a sample of 60 males (19 C, 21 L, and 20 W).

Mean body mass did not differ between males assigned to the C ($0.73 \pm 0.03 \text{ g}$, $n = 21$), L ($0.73 \pm 0.02 \text{ g}$, $n = 29$), and W ($0.76 \pm 0.03 \text{ g}$, $n = 29$) treatments (ANOVA, $F_{2, 75} = 0.76$, $p = .47$). We found no effect of treatment (C, L, and W) ($\chi^2_2 = 1.16$, $p = .56$), spermatophore order (pre- and post-fight) ($\chi^2_1 = 0.69$, $p = .41$), or their interaction ($\chi^2_2 = 0.55$, $p = .75$) on sperm viability (Figure 1). Estimated effect sizes and 95% CI around the mean of predictors are reported in Table S1 (see online Data S1). These values show that the point estimates are centered on zero and that credible intervals are relatively symmetric, implying this was a null result rather than lack of power to detect an effect.

4 | DISCUSSION

We show for male field crickets that the experience of winning or losing a fight does not lead to short-term adjustment of sperm viability. There are several explanations for such lack of plasticity. First, male fighting ability and hence the outcome of the fight needs to be a reliable indicator of sperm competition risk and future mating prospects to trigger any strategic response (delBarco-Trillo, 2011; Kelly & Jennions, 2011). Experiencing a single fight may not sufficiently signal future sperm competition and elicit a response compatible with the perceived costs of future matings, which may instead require additive effects of multiple fights (Hsu & Wolf, 1999). One of our recent studies indeed shows that over multiple fights, males reduce their aggressiveness while increasing sperm production (Tuni, Han, & Dingemans, 2018). This suggests that males may respond to the enhanced sperm competition perceived through multiple fights by enhancing fertilization abilities (sperm numbers) rather than allocating to winning fights (aggressiveness). Furthermore, the asymmetries in reproductive success between winners and losers should be high enough to maintain ejaculate tailoring. If dominant contest winners are not efficient in monopolizing females and/or the proportion of losers sneaking copulation increases in the population, sperm competition increases for winners. In such conditions, males would

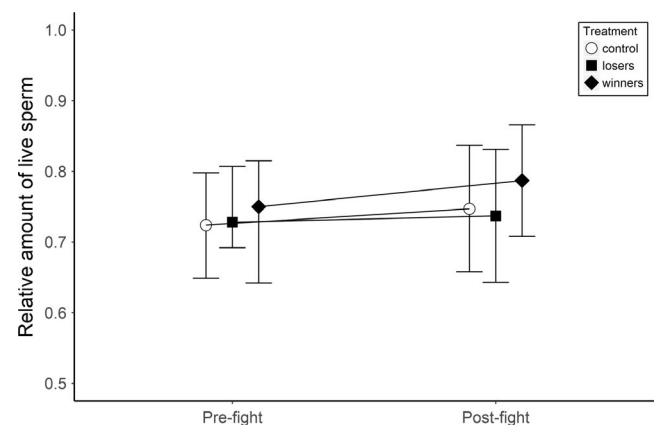


FIGURE 1 Sperm viability (proportion of live sperm cells) in pre-fight and post-fight spermatophores of males assigned to fighting trials, either winning (filled circle) or losing (filled triangle), and to control trials (empty square) with no fighting experience

respond to the average sperm competition experienced, with winners and losers releasing ejaculates of similar quality (Parker, 1990b). A field study on another gryllid (*Gryllus campestris*), for example, reports limited effectiveness of pre-mating male-male competition for avoiding post-mating sperm competition (Fisher, Rodríguez-Muñoz, & Tregenza, 2016), highlighting the importance of understanding intra-sexual competitive dynamics in the wild. Second, ejaculate tailoring may be only necessary if males are sperm-limited. The energetic resources needed to package sperm are seemingly not a limiting factor for *Gryllus bimaculatus* males that can quickly replenish sperm stores in-between matings (i.e., initiate production of a new spermatophore within 6 min from a copulation) (Ootsubo & Sakai, 1992), and discard spermatophores frequently and independently of copulation (Kumashiro, 2003). Finally, in natural conditions males may already possess previously formed spermatophores during same-sex interactions, leaving a little scope for post-fight ejaculate adjustment.

In light of the existing literature revealing differences in sperm quality between dominant-winners and subordinate-losers field crickets (Thomas & Simmons, 2009; Tunı et al., 2016), our findings indirectly point to intrinsic differences in ejaculates between males of different social status rather than ejaculate tailoring derived from fighting. Dominance in male crickets is generally associated with larger body size (Simmons, 1986; Tunı et al., 2018), despite size not being an absolute predictor for winning a fight (Hofmann & Schildberger, 2001). Dominant males are preferred by females (Simmons, 1986), possess higher immune responses (Rantala & Kortet, 2004), suggesting that fighting ability is correlated to a higher male phenotypic condition. In systems where males face contest competition, the increase in energetic allocation to traits used in fights, such as weapons or body size, is often coupled with a reduction in the energy allocated to traits used for fertilizations, such as testes size or sperm traits (Simmons et al., 2017). The lower sperm quality observed in male *G. bimaculatus* that won a fight (Tunı et al., 2016) may hence result from trade-offs in resource allocation between fighting and fertilization abilities. Worthington, Gress, Neyer, and Kelly (2013) came to similar conclusions when investigating sperm allocation in the house cricket *Acheta domesticus*, as they found smaller males, which occupy a disadvantaged role in mating, having sperm of higher viability (Worthington et al., 2013).

Finally, we have targeted sperm viability as it is central in determining fertilization success in crickets (García-González & Simmons, 2005), but obviously, we cannot exclude that males would have responded to the social interaction modifying other sperm traits, such as sperm numbers (Tunı et al., 2018).

To conclude, we show that males do not change their ejaculate quality in response to the outcome of a single fight. Differences in other attributes between winner and losers, such as body condition, may instead drive long-term developmental changes in sperm production, for example, affecting spermatogenesis. These, and not the experience of a fight per se, may explain the differences in sperm quality previously described in this system.

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CONFLICT OF INTEREST

Authors declare no conflict of interests.

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SUPPORTING INFORMATION

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