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The cost of aggression in an animal without weapons

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Abstract

To understand the prevalence and conditional use of aggression among animals, one has to know its costs and benefits. The obvious cost of aggression in animals that possess teeth, claws or other specialized weaponry is injury. Many species, however, do not have such body parts and thus cannot readily injure others. The cost of aggression in these animals is not well studied. We tested whether aggression has a fitness cost in fruit flies, which can serve as a model species for animals without weapons that engage in aggression. In three experiments employing distinct protocols, we allowed focal flies to fight for control of an attractive food patch over 4 days and then compared their survivorship to that of flies not engaged in conflict. In all three experiments, fly survivorship was lower in the aggression than no-aggression treatments. Microscopic examination revealed no differences in wing damage between flies of the aggression and no-aggression treatments. The two most likely, non-mutually exclusive explanations for lower survivorship post-fighting are physiological changes due to stress, and metabolic alterations associated with a life-history strategy optimized for high-conflict settings.

KEYWORDS

aggression, fighting, stress, survivorship

1 | INTRODUCTION

Aggression is prevalent among animals including humans and has been subjected to numerous enlightening evolutionarily-informed analyses (Archer, 1988; Darwin, 1871; Huntingford & Turner, 1987; Maynard Smith, 1982; Pinker, 2011; Wilson, 1975). To understand the occurrence and conditional use of aggression between and within species it is essential to quantify its benefits and costs. Some of these benefits and costs are obvious. In many species, aggression is essential for securing mates, protecting self and offspring, and attaining key resources such as food and shelter (Archer, 1988; Darwin, 1871; Huntingford & Turner, 1987; Wilson, 1975). For example, in the southern elephant seal (*Mirounga leonina*), only the largest males are harem holders. These males mate with the majority of females and prevent subordinate males from mating primarily through threats and rarely via fights (McCann, 1981). Consequently, the harem holders father approximately 90% of the offspring (Fabiani, Galimberti, Sanvito, & Hoelzel, 2004).

The most obvious costs of aggression are injury and death (Archer, 1988; Huntingford & Turner, 1987). For example, 95% of male cervids older than 2.5 years inspected in Western Canada had scars from combat injuries (Geist, 1986). Lethal aggression is prevalent among a wide variety of animals including hymenopterans, birds and mammals (Gómez, Verdú, González-Megías, & Méndez, 2016; Matthews, González, Matthews, & Deyrup, 2009; Piper, Walcott, Mager, & Spilker, 2008; De Vita, 1979). For example, in the California harvester ant (*Pogonomyrmex californicus*), contests lasting more than 1 min between two workers from different colonies resulted in one fatality in 57% of the cases and both workers dying in 29% of the fights (De Vita, 1979).

While many animals can inflict significant damage on others by using teeth, claws or specialized weapons (Darwin, 1871; Hardy & Briffa, 2013; Emlen 2014), a large proportion of species are unarmed. Aggressive encounters, however, are still prevalent in such species. Well-studied examples include butterflies and damselflies (Davies, 1978; Kemp, 2013; Kemp & Wiklund, 2001; Marden & Waage, 1990). The costs of aggressive encounters in unarmed

animals, however, are not clear. It is possible that costs are negligible. Alternatively, one can invoke the presumed default costs of time and energy (Archer, 1988; Maynard Smith & Price, 1973). Other possible costs in unarmed species include stress, poor health and increased exposure to predators (Huntingford & Turner, 1987; Riechert, 1988; Sapolsky, 2005). Nevertheless, we have relatively little experimental data about the cost of aggression in unarmed species.

Among unarmed species, fruit flies (*Drosophila melanogaster*) stand out as a superb model system because of their suitability for integrative research on the mechanisms, ecology and evolution of aggression (Asahina, 2017; Baxter, Barnett, & Dukas, 2015; Chen, Lee, Bowens, Huber, & Kravitz, 2002). The benefits of fruit fly aggression are clear. In settings with small fruits and low fly density, capable males defend attractive fruits and mate with the females that arrive to feed and lay eggs (Hoffmann, 1987; Markow, 1988). The males also guard the females against intruding males (Baxter et al., 2015). At high densities or in settings with large fruits, where males directly pursue females, the males rely on subtle aggression to interfere and take over courtship from their rivals (Baxter, Mentlik, Ieta, & Dukas, 2018). Consequently, aggression is positively associated with fitness in male fruit flies (Baxter et al., 2015, 2018).

The most common components of fruit fly aggression include wing threats, where a fly raises its wings at 45° towards its opponent, chasing and lunging, in which the aggressor hits its opponent. On rare occasions, flies also engage in boxing or tussling (Chen et al., 2002). Even without weapons, one might surmise that hitting, boxing and tussling can inflict damage to the body, especially the delicate wings. In fact, Davis, Thomas, Liu, Campbell, and Dierick (2018) relied on wing damage for screening fly lines that were hyperaggressive. Another experiment, however, documented no difference in wing damage throughout the lifespan of males that were either housed alone or with rivals (Bretman, Westmancoat, Gage, & Chapman, 2013). Hence, the issue of bodily damage requires further research. An alternative, relevant and comprehensive way of assessing costs of aggression is to compare the survivorship of focal males that engage in aggression for some time to that of control males. Indeed a variety of studies in fruit flies and other species assessed pertinent fitness costs of different traits through measuring survivorship (Bretman et al., 2013; Gendron et al., 2014; Mery & Kawecki, 2005; Moret & Schmid-Hempel, 2000).

We conducted a set of experiments to quantify the cost of aggression in fruit flies. Specifically, we tested the predictions that aggression will lead to lower survivorship and higher wing damage. Our general protocol was to expose focal flies to aggression for 4 days and then quantify their survivorship. There are different ways of measuring survivorship and we chose two of them, survivorship with no food and survivorship with neither food nor water. Such measures have been used successfully in previous studies (Gendron et al., 2014; Mery & Kawecki, 2005; Moret & Schmid-Hempel, 2000). Our rationale was that we wished to measure the immediate, short-term effects of aggression on subsequent survivorship. We could not provide daily controlled aggression over months and, in any event, fruit flies' expected survivorship in nature is only several days (Baxter & Dukas, 2017; Rosewell & Shorrock, 1987). While reduced survivorship under starvation could

reveal possible metabolic costs of aggression, reduced survivorship under both starvation and desiccation might also hint at subtle injuries, which increase haemolymph loss (Sepulveda et al., 2008).

Our basic protocol included an aggression treatment, in which focal males interacted with aggressive males, and a control treatment, where males were alone. To control for the known negative effects of females on survivorship (Cordts & Partridge, 1996; Gendron et al., 2014; Partridge & Farquhar, 1981), we included no females in the experiments. While the purest way to prevent male aggression is to isolate each male, we still had to assess the possibility that survivorship was merely affected by the presence of another male rather than by aggression. It is indeed known that males alter their behaviour and physiology in the presence of rivals and that this might affect their survivorship (Bretman, Fricke, & Chapman, 2009; Bretman et al., 2013). To address this issue, we conducted another experiment in which we housed focal males of the control treatment with young, 24-hr-old males, who show little aggression (Baxter & Dukas, 2017). Finally, in addition to the survivorship experiments, we also quantified wing damage of focal flies of the aggression and control treatments.

2 | METHODS

2.1 | General

We housed all flies in population cages in an environmental chamber at 25°C and 60% relative humidity with a 12:12 hr light:dark cycle with lights on at 10 a.m. and reared the experimental flies at a low density of approximately 300 eggs per 240 ml bottle containing 50 ml of standard fly medium made of water, sucrose, cornmeal, yeast, agar and methyl paraben. Our focal flies were descendants of wild-caught *D. melanogaster* captured in several sites in Southern Ontario in summer 2014 and kept in a large, outbred laboratory population (Baxter & Dukas, 2017). In all of the experiments, focal males of the aggression treatment interacted with males from a hyperaggressive line generated via artificial selection and inbreeding in the Kravitz laboratory at Harvard University (Chowdhury, Chan, & Kravitz, 2017; Penn, Zito, & Kravitz, 2010). We used the hyperaggressive males as the stimulus flies to ensure that we had sufficient levels of aggression in all the experimental arenas. Note that the hyperaggressive flies served only as stimulus flies because they do not represent a naturally occurring phenotype. Our research complied with all applicable laws and did not require approval from an ethics committee.

2.2 | Survivorship following aggression

We conducted three experiments to test whether aggression is associated with reduced survivorship. All three experiments had the same general protocol, and each experiment had two successive replicates. While we aimed to have 128 flies in each experiment, our final sample sizes were sometimes lower due to fly escape. Our experimental arenas consisted of petri dishes 35 mm in diameter and 8 mm high. To prevent flies from walking on the ceilings, we coated them with a slippery coating of Surfasil (Sigma Aldrich). We covered the floor of each arena with filter paper and placed at its centre a circular food

patch made of standard medium and measuring 1.3 cm in diameter and 1.5 mm high. At the middle of the food patch, we dispensed approximately 25 mg of a thick suspension made of 5 g live yeast in 10 ml grapefruit juice to create a ball 3 mm in diameter. This combination is highly attractive to fruit flies and effective at eliciting aggression.

2.3 | Experiment 1: survivorship in agar vials (starvation)

On day 1, we sexed by aspiration the focal, wild-type males within 8 hr of eclosion and placed each male alone inside a standard 40 ml vial containing 5 ml medium. On days 1–4, we sexed newly eclosed hyperaggressive males and placed them individually in food vials. On day 5 at 8 a.m., we placed each 4-day-old focal male of the aggression treatment with a 4-day-old hyperaggressive male inside each arena. The hyperaggressive males were marked with pink fluorescent powder (BioQuip), which does not affect fly behaviour and aggression (Baxter & Dukas, 2017; Crumpacker, 1974). At the same time, we also placed each 4-day-old focal male of the no-aggression treatment alone inside each arena. We kept the flies in the arenas for 8 hr as this period represented a realistic duration of daily aggressive interactions (Figure 1a). Then, at 4 p.m., we moved each focal male back to its food vial and returned all focal males to the environmental chamber. We also discarded the hyperaggressive males and cleaned the arenas. On day 6 at 8 a.m., we again placed each focal male of the aggression treatment in a fresh arena with a new 4-day-old hyperaggressive male, and each male of the no-aggression treatment alone in a fresh arena. We repeated the same protocol again on days 7 and 8 so that each focal male had a total of four periods of interactions with four distinct hyperaggressive stimulus males. We used fresh stimulus males every day to eliminate effects of their experience over successive days.

To help us interpret the survivorship data, we conducted video recordings followed by detailed analyses of fly behaviour. Specifically, we recorded 8 arenas of the no-aggression and 7 arenas of the aggression treatment for 15 min at 8:00 a.m. and 15 min at 1:00 p.m. on each of the 4 days of the experiment. Later, an observer blind to fly treatment, day and time of day recorded the total duration of aggressive behaviour by the focal and hyperaggressive males (Baxter & Dukas, 2017) using the BORIS software (Friard & Gamba, 2016). Aggressive behaviour included wing threats, where a fly raises its wings at 45° towards its opponent, chasing and lunging, in which the aggressor hits its opponent, and rare incidents of boxing and tussling (Chen et al., 2002). We conducted two analyses, one including the duration of all aggressive behaviours and the other focusing only on lunges. While we prefer to analyse total aggression, we also present the separate lunge analysis because many fruit fly aggression studies focus on lunges. We analysed the data using R (R-Core-Team, 2015) with a generalized linear mixed-effects model within the R package “lme4” (Version 1.1–18–1) with Gamma distribution for the aggression duration, which is conveniently described by gamma distribution, and Poisson distribution for the lunge frequency. We included treatment, day and time of day as fixed effects and male ID as a random effect.

At 4 p.m. on day 8, we moved each focal male into a short snap-cap vial (22 mm diameter × 48 mm long) containing 4 ml agar, which provided a source of water. The lids of the snap-cap vials had a small pinhole for ventilation. We randomly placed the vials vertically into *Drosophila* activity monitors (Trikinetics Inc.; software version 3.08) positioned inside opaque plastic containers (l × w × h: 51.5 × 36 × 41 cm) that were humidified at ~75% RH and kept at 25°C. We placed an LED lightbulb on the standard light:dark cycle over a hole in the centre of each container lid to illuminate the monitors from above. The activity monitors continuously recorded fly movement until all flies died. Then an observer blind to fly treatment inferred fly survivorship based on the time of last activity. In a preliminary study, we found that survivorship inferred from the activity monitors closely matched survivorship directly recorded by observers.

Overall, our protocol simulated a realistic natural scenario where males either interacted with other males or remained alone on a food patch for a daily activity period of 8 hr each day for 4 days before we tested their survivorship. We ran 2 replicates of this experiment, with a total sample size of 63 focal flies in the aggression treatment and 63 focal flies in the no-aggression treatment. We analysed the survivorship data with a Cox proportional hazards regression model within the R “survival” package (Version 2.41-3), with treatment and replicate as fixed effects. Our data for all three experiments met the model assumptions.

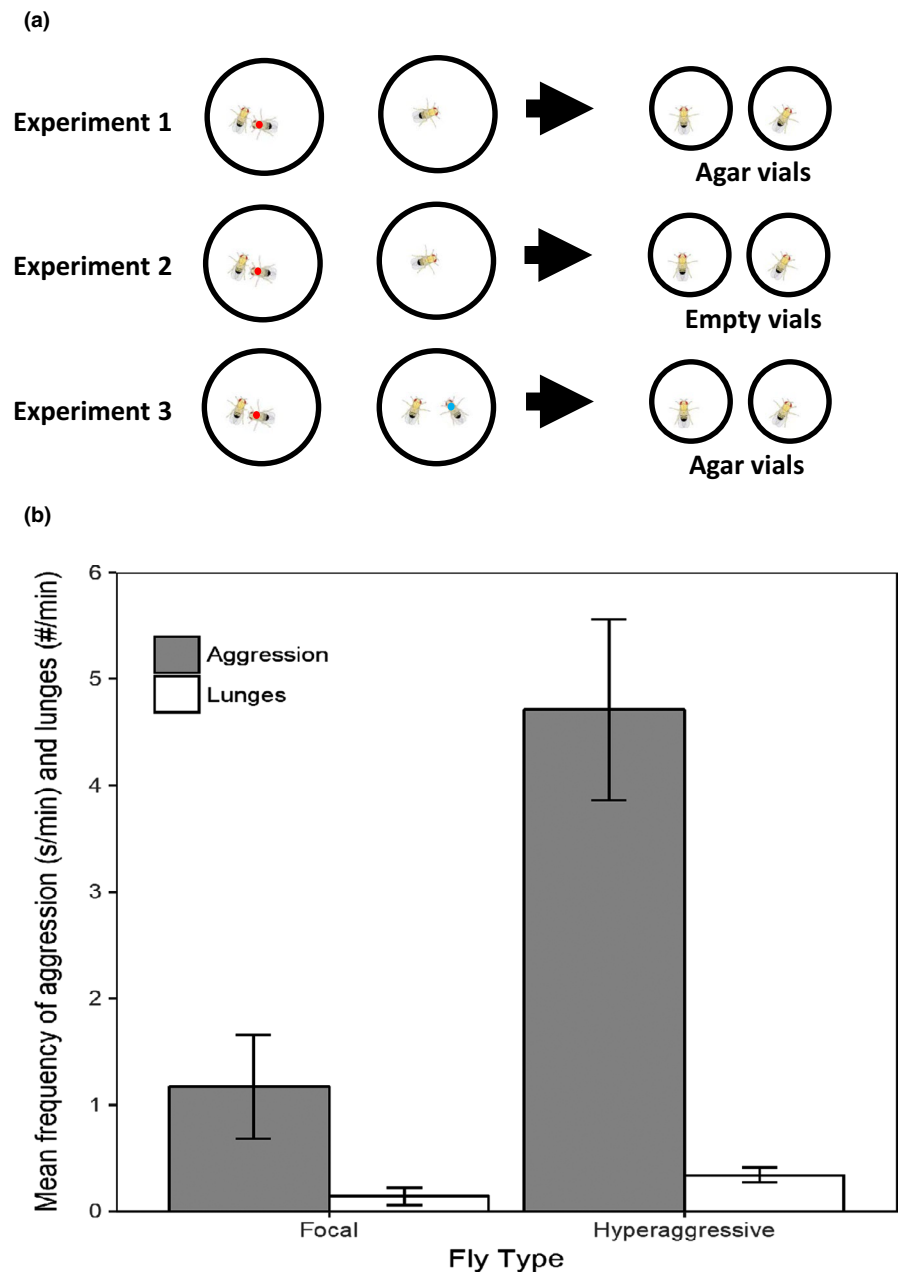
2.4 | Experiment 2: survivorship in empty vials (desiccation)

While reduced survivorship under starvation could reveal possible metabolic costs of aggression, reduced survivorship under both starvation and desiccation might also hint at subtle injuries, which increase haemolymph loss (Sepulveda et al., 2008). To address this issue, we conducted an experiment nearly identical to the one above except that, on day 8 at 4 p.m., we placed each focal male in an empty snap-cap vial. That is, the males had neither food nor water. We ran 2 replicates of this experiment, with a total sample size of 64 focal flies in the aggression treatment and 64 focal flies in the no-aggression treatment.

2.5 | Experiment 3: survivorship after interacting with either hyperaggressive or docile males

This experiment was identical to experiment 1 with one major difference. Here the no-aggression focal flies were not alone. Rather, each no-aggression focal fly interacted for each of the 4 days with a young, 24-hr-old male from the wild-derived population. The young males were marked with pink fluorescent powder for identification. Young males show little aggression (Baxter & Dukas, 2017). We ran 2 replicates of this experiment, with a total sample size of 64 focal flies in each treatment. To assess the level of aggression in the aggression and no-aggression treatments, an observer blind to fly identity recorded aggression bouts in each arena for 5 min on the morning of day 8 of replicate 2. These observations included 38 and 37 males of the aggression and no-aggression treatments, respectively.

FIGURE 1 (a) The experimental protocol in the three survivorship experiments. In all three experiments, each focal male of the aggression treatment spent 8 hr a day for 4 days interacting with a hyperaggressive male (depicted with a red dot). Each focal male of the no-aggression treatment was alone in experiment 1 (starvation) and experiment 2 (desiccation), and with a docile male in experiment 3 (depicted with a blue dot). (b) The average frequency (mean \pm SE) of all aggressive behaviours (wing threats, chasing, lunging and rare incidents of boxing and tussling) and the number of lunges by focal and hyperaggressive males during the 15 min of observation periods [Colour figure can be viewed at wileyonlinelibrary.com]



2.6 | Experiment 4: wing damage following aggression

In a preliminary experiment, we closely examined through a microscope flies subjected to aggression over 4 days and searched for any sign of damage while focusing especially on the antennae, wings and bristles throughout the body. While we found significant wing damage, we noted almost no antennal or bristle damage. We thus subsequently focused only on wing damage. We conducted an experiment identical to experiments 1 and 2, in which focal males in the aggression treatment interacted with hyperaggressive males while focal males of the no-aggression stayed alone in arenas for 4 days. At the end of the experience phase at 4 p.m. on day 8, an experienced observer blind to fly treatment thoroughly examined each fly's wings under a microscope and noted damage. The observer categorized wing damage into three

distinct categories: (a) small nicks, (b) small pieces missing and (c) large wing portions absent. We had 38 and 37 males in the aggression and no-aggression treatments, respectively. We analysed the presence of damage data (damage or no damage) with a Pearson's Chi-squared test, and the damage-size data with a Mann-Whitney *U* test.

3 | RESULTS

3.1 | Survivorship following aggression

3.1.1 | Experiment 1: aggression and subsequent survivorship in agar vials (starvation)

Our video analyses of focal and hyperaggressive males during 15 min observations on the 4 days of the aggressive interactions indicated

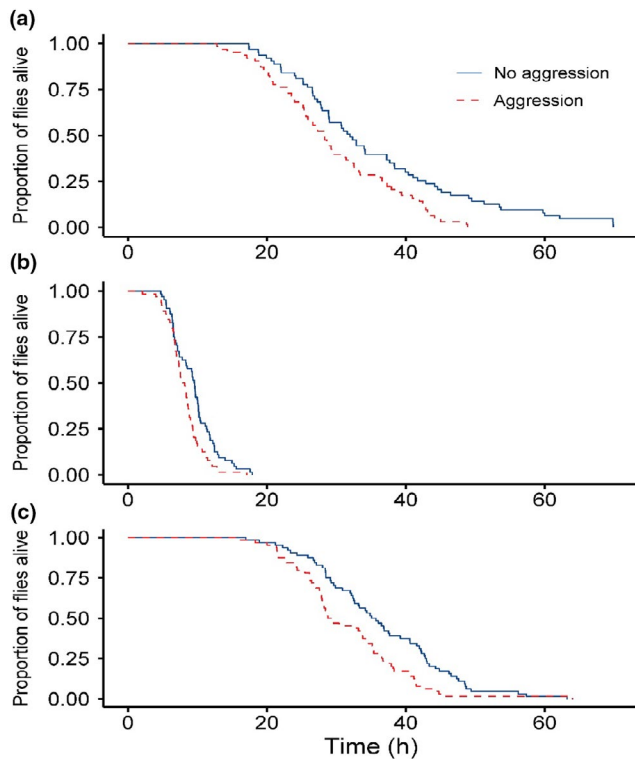


FIGURE 2 Survivorship of males of the aggression and no-aggression treatments in each of the three experiments. Each male of the no-aggression treatment was alone in (a) experiment 1 (starvation) and (b) experiment 2 (desiccation). In experiment 3 (c), each male of the no-aggression treatment was with a docile male. Sample sizes were 63 per treatment in (a) and 64 per treatment in (b) and (c) [Colour figure can be viewed at wileyonlinelibrary.com]

that, while both male types showed persistent aggression, the hyper-aggressive males were significantly more aggressive ($\chi^2_1 = 53.4$, $p < .01$; Figure 1b) and delivered more lunges ($\chi^2_1 = 57.4$, $p < .01$; Figure 1b) than the focal males. Day and time of day effects were significant for lunges (day: $\chi^2_3 = 95$, $p < .01$; time of day: $\chi^2_1 = 5.7$, $p < .05$). Time of day did not significantly affect aggression duration ($\chi^2_1 = 0.60$, $p = .44$) while day had a significant effect ($\chi^2_3 = 12.60$, $p < .01$). Focal males of the aggression treatment had significantly lower survivorship than focal males of the no-aggression treatment (Figure 2a; $Z_1 = 4.4$, $p < .001$ for treatment effect and $Z_1 = -6.6$, $p < .001$ for replicate effect).

3.1.2 | Experiment 2: survivorship in empty vials (desiccation)

Focal males of the aggression treatment had significantly lower survivorship than focal males of the no-aggression treatment (Figure 2b; $Z_1 = 2.8$, $p < .001$ for treatment effect and $Z_1 = -0.8$, $p = .5$ for replicate effect).

3.1.3 | Experiment 3: survivorship after interacting with either hyperaggressive or docile males

Focal males housed for 4 days with hyperaggressive males had significantly lower survivorship than focal males housed for 4 days with

1-day-old males (Figure 2c; $Z_1 = 3.1$, $p < .01$ for treatment effect and $Z_1 = 3.3$, $p < .01$ for replicate effect). Our behavioural observations indicated that, in both treatments, focal males showed little aggression (3% of the arenas in the aggression treatment and 6% of the arenas in the no-aggression). On the other hand, the average number of aggressive bouts by hyperaggressive and docile, young wild-type males was 3.9 ± 0.6 versus 0.7 ± 0.3 , respectively (Mann-Whitney Test, $U = 970$, $p < .01$).

3.1.4 | Experiment 4: wing damage following aggression

A similar proportion of flies showed wing damage in the aggression and no-aggression treatments (0.37 and 0.32, respectively; $\chi^2_1 = 0.2$, $p = .6$). The magnitude of wing damage was also similar in the aggression and no-aggression treatments (Mann-Whitney $U = 76$, $n = 14$ and 12, $p = .7$).

4 | DISCUSSION

Our major finding was that flies engaged in conflict consequently had lower survivorship than flies that did not experience aggression. We found similar results when we tested fly survivorship without food (Figure 2a) and with neither food nor water (Figure 2b). Furthermore, flies of the aggression treatment had lower survivorship both when compared to no-aggression flies housed alone (Figure 2a,b) and when compared to no-aggression flies housed with docile males (Figure 2c). Finally, flies of the aggression treatment sustained no more wing damage than no-aggression flies. Previous analyses of aggression in animals without weapons assumed the standard costs of energy and time (Archer, 1988; Brown, 1964; Kemp & Wiklund, 2001; Maynard Smith & Price, 1973). By measuring survivorship, we could link aggression in an unarmed species to an explicit fitness cost. Our results are consistent with data indicating that male fruit flies from lines selected for territoriality had lower survivorship than males of control lines (Hoffmann & Cacoyianni, 1989).

We chose to quantify survivorship under the two conditions of food deprivation only and withdrawal of both food and water. We assumed that subtle injuries might increase water loss from the body and hence lead to a larger treatment effect under desiccation plus starvation than under starvation only (see Sepulveda et al., 2008). We found, however, no evidence for such greater effect of aggression under desiccation plus starvation than starvation (Figure 2, panels a versus b). This could indicate that either aggression was not associated with a higher frequency of injuries or that such injuries are associated with both water and nutrient loss. Alternatively, aggression might lead to increased need for both water and nutrients.

In addition to employing two protocols for testing survivorship, we also used two control treatments. The best control for the aggression treatment was housing males without rivals to ensure that no aggression could occur. This, however, left open the possibility that it is the presence of another male rather than aggression per se

that leads to differential survival. We addressed this issue by housing focal males of the no-aggression treatment with docile males even though we could not fully eliminate aggression in this treatment. Our results indeed indicated that we had high and low aggression treatments and that this test yielded survivorship patterns similar to those in the comparison between males housed with hyperaggressive males versus males kept alone (Figure 2c).

While we cannot link specific mechanisms to the lower survivorship of flies engaged in aggression, the most likely explanation is a broad spectrum of biological changes associated with adjustments to high-conflict settings. Such changes typically prioritize metabolic pathways that maximize current reproductive success at the cost of reduced long-term survival. At the ultimate level, the life-history trade-off between investment in current reproduction versus future survival and reproduction is well established (Roff, 1992; Stearns, 1992). At the proximate level, there is vast endocrinological and epidemiological literature on stress, its short-term benefits as well as adverse long-term outcomes (Gesquiere et al., 2011; Korte, Koolhaas, Wingfield, & McEwen, 2005; McEwen & Wingfield, 2003; Sapolsky, 2005; Steptoe, Shankar, Demakakos, & Wardle, 2013).

Adjustments to adverse, highly competitive settings may include mobilization of energy reserves and physiological pathways that enable long periods of high levels of activity associated with aggressive interactions and the rapid recovery following fighting (Briffa & Sneddon, 2007; Marden & Waage, 1990). Dominant males must remain vigilant in order to fight new challengers and repel sneaking males, while subordinate males might adopt other, yet equally demanding, strategies for fleeing from dominant males and seeking alternative mating opportunities (Gesquiere et al., 2011; Sapolsky, 2005). In fruit flies, males that defend attractive resources and guard females are indeed highly active and more aggressive than non-guarding males, and subordinate males appear to seek mating opportunities through sneaking when dominant males are less vigilant or resting (Baxter et al., 2015, 2018). Furthermore, males reared with other males subsequently mate for longer durations than males reared alone, in anticipation of sperm competition (Bretman et al., 2009). Finally, males reared with other males have lower survivorship than males reared alone (Bretman et al., 2013).

While our experiments have focused on males, previous research on female fruit flies documented reduced female survivorship in response to male harassment. That is, in addition to the known harmful effects of matings, male presence alone had negative impacts on female fitness (Partridge & Fowler, 1990). Indeed, a comparison of 24 experiments quantifying the overall fitness cost of female exposure to males with the cost of a single mating suggested that approximately 70% of the overall fitness cost to females is attributed to the detrimental effects of evading pursuing males, which include behaviours such as running, flying and kicking (Kuijper, Stewart, & Rice, 2006; Rice et al., 2006). These data agree with our results, which link antagonistic interactions to reduced survivorship.

A few studies have showed that even cues alone without adversarial interactions can alter metabolic changes that lead to reduced lifespan. Most notably, male fruit flies exposed to female sex pheromones rapidly decreased fat stores, had lower resistance to

starvation and shorter life span than control males. Surprisingly though, these effects could be reversed by mating (Gendron et al., 2014; Harvanek et al., 2017), which blurs the adaptive significance of such change. Similarly, fruit flies exposed to odours from live yeast altered metabolic activity and had shorter lifespan than control flies (Libert et al., 2007; Poon, Kuo, Linford, Roman, & Pletcher, 2010). Olfactory and gustatory perception also regulates lifespan in *C. elegans* (Alcedo & Kenyon, 2004; Apfeld & Kenyon, 1999) suggesting that conserved pathways modulate senescence in response to pertinent cues.

Although fruit flies are unarmed, lunging, boxing and tussling can result in wing damage. Our results, however, did not reveal higher wing damage in males of the aggression than no-aggression treatments. In an experiment spanning over a month, wing damage in fruit flies was not significantly higher in the treatments in which each male was held with another male than in treatments where each male was held without a rival (Bretman et al., 2013). On the other hand, when fruit fly lines that vary in aggression were held in groups of 15 males per vial for 3 weeks, there was a positive correlation between a line's aggression score and wing damage (Davis et al., 2018). While the disparate data come from different fly lines and distinct protocols, it is fair to conclude that we cannot reject the possibility that aggression, even in unarmed fruit flies, may cause rare but costly injuries under realistic settings.

Overall, we have documented a clear cost of aggression in an animal without weapons. The reduced survivorship in flies engaged with aggression is most likely due to physiological changes associated with high-conflict settings. Such changes, which are typically linked to stress, are prevalent among many species including humans. The abundance of established tools and protocols available for studying fruit flies makes them an ideal model system for further research on the mechanisms and evolutionary biology of adjustments to high-stress conditions.

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CONFLICT OF INTEREST

None.

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