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Assessment strategies and fighting patterns in animal contests: a role for serotonin?

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Abstract

Accurate assessment of the probability of success in an aggressive confrontation with a conspecific is critical to the survival and fitness of the individuals. Various game theory models have examined these assessment strategies under the assumption that contests should favor the animal with the greater resource-holding potential (RHP), body size typically being the proxy. Mutual assessment asserts that an individual can assess their own RHP relative to their opponent, allowing the inferior animal the chance to flee before incurring unnecessary costs. The model of self-determined persistence, however, assumes that an individual will fight to a set personal threshold, independent of their opponent's RHP. Both models have been repeatedly tested using size as a proxy for RHP, with neither receiving unambiguous support. Here we present both morphological and neurophysiological data from size-matched and mismatched stalk-eyed fly fights. We discovered differing fighting strategies between winners and losers. Winners readily escalated encounters to higher intensity and physical contact and engaged in less low-intensity, posturing behaviors compared with losers. Although these fighting strategies were largely independent of size, they were associated with elevated levels of 5-HT. Understanding the neurophysiological factors responsible for mediating the motivational state of opponents could help resolve the inconsistencies seen in current game theory models. Therefore, we contend that current studies using only size as a proxy for RHP may be inadequate in determining the intricacies of fighting ability and that future studies investigating assessment strategies and contest outcome should include neurophysiological data.

Key words: aggression, assessment, fighting strategies, serotonin, monoamines, resource-holding potential.

Engaging in aggressive conflicts can be risky, with the potential of serious injury or death. Although not all conflicts result in injury, with some fighting styles being incapable of inflicting physical damage, prolonged aggressive posturing can waste time and energy, especially if unsuccessful, as well as increase exposure to predators. For these reasons and others, animals have developed impressive, sometimes complex, fighting strategies and signaling mechanisms to resolve conflicts. Signals exchanged during aggressive interactions are of great functional importance because they mediate access to resources while minimizing the costs associated with fighting (Geist 1966; Emlen 2008). Body size relationships, in particular, exert a strong influence on contest outcome, and many signals appear to function to advertise individual size.

Many theoretical models that have been developed to determine contest outcome use body size as the primary indicator of an animal's fighting ability, typically termed resource-holding potential (RHP). For example, the model of mutual rival assessment predicts that opponents assess each other's relative body size as a proxy for

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RHP to aid in the decision to either fight or flee (Parker 1974; Smith and Parker 1976). This assessment provides the smaller opponent, typically inferior in fighting ability, the opportunity to avoid a potentially costly contest in which they have a low probability of success. In contrast, the model of self-determined persistence proposes that the individual engages in a contest to a set personal threshold, hypothesized to be dictated by their own size, independent of their opponent's size (Taylor and Elwood 2003).

Both models predict that as the size disparity between opponents increases the contest duration will decrease, because the bigger opponent will always fight for longer regardless of whether its size is being appraised by the smaller individual. However, Taylor and Elwood (2003) suggest that separate regressions of contest duration against either the size of the loser or against the size of the winner can distinguish between the 2 models. With mutual rival assessment, the regression coefficients of winner and loser size should be similar in magnitude but opposite directions, negative for winners and positive with losers (Arnott and Elwood 2009). In contrast, the self-determined persistence model predicts that the coefficients will be characterized by a significant positive relationship with loser size and a weakly positive relationship with winner size (Arnott and Elwood 2009). However, neither model, which represents the 2 extremes of animal assessment strategies, receives unambiguous support. Experimental tests of the predictions of each of the models have yielded inconsistent or contradictory results (Morrell et al. 2005; Stuart-Fox 2006; Brandt and Swallow 2009). A more realistic "partial mutual assessment" strategy in which individuals have reliable knowledge of themselves but limited information about their opponent may better capture the strategy actually employed during assessment (Prenter et al. 2006).

We contend that empirical attempts to gauge the validity of different assessment strategies using only body size as a proxy measure of RHP and contest duration as a measure for cost may be overly simplistic and inadequate to the task. Contest duration alone may be a poor representation of the cost of a contest given the variability in the types of displays or interactions that take place, e.g., 10 min of low-intensity (LI; non-physical) behaviors will have a much lower associated cost compared with 10 min of high-intensity (HI) behaviors. Similarly, size alone does not appear to sufficiently capture the intricacies of individual fighting ability (i.e., motivation, experience, etc.) and should be just 1 factor used to assess and understand contest outcomes. This is especially the case in contests that are resolved predominantly through signal exchange rather than physical infliction of injury. Given the complex behaviors and signals animals engage in during contests, it is reasonable to suggest that they are capable of transmitting and receiving more information about RHP than simply body size that will determine how willing each opponent is to actually commit to fighting.

The neural, sensory, and cognitive mechanisms that may permit assessment not only of rival size but also other factors associated with RHP remain largely unexplored and have the potential to resolve inconsistencies predicted from simple models of contest outcome that rely only on body size. Many of the non-morphological factors responsible for mediating aggressive contests involve an altered motivational state, most likely facilitated by neurobiological factors such as biogenic amines (Bubak et al. 2014a). For example, octopamine (OA) has been demonstrated to be a key element in the rewarding experience of territory possession in crickets, with previously defeated individuals pharmacologically diminished of OA losing the aggressive enhancing effect of occupying a shelter (Rillich et al. 2011). Brain serotonin (5-HT) in invertebrates has been implicated in both overall elevated aggression, including higher intensity aggressive behaviors and escalation patterns, as well as reduced willingness to retreat (Huber et al. 1997; Bubak et al. 2014a, 2014b). Altering brain concentrations of certain biogenic amines in 1 opponent of size-matched pairs has been shown to significantly influence the progression and outcome of fights in several invertebrate species (for review, see Bubak et al. 2014a). Individual differences in levels of biogenic amines between opponents could account for some of the unexplained variation in contest outcome seen in experiments that only use size as a proxy for RHP.

Obtaining morphological, behavioral, and neurophysiological data in a single species can be difficult. However, to understand the intimate interactions between morphology and neurophysiology and how this relationship directs behavioral output, experiments that can simultaneously account for all of these variables need to be conducted. Stalk-eyed flies (Diopsidae: *Teleopsis dalmanni*) provide such a model species, where researchers can incorporate selective neurochemical detection and manipulation techniques while simultaneously assessing individual variance in both morphology and behavior. This species is characterized as having eye bulbs displaced laterally on long stalks, which males use as aggressive signals during confrontations over mates and food resources (Wilkinson and Dodson 1997; Wilkinson and Johns 2005). Males follow a stereotyped escalation pattern starting with lining up of eyestalks, progressively moving toward more intense behaviors (de la Motte and Burkhardt 1983; Panhuis and Wilkinson 1999). Because eye span is an accurate indicator for size in males, individuals with longer eye span typically defeat smaller males (Burkhardt and de la Motte 1983, 1987; Small et al. 2009; Egge and Swallow 2011). However, although size is a significant factor, smaller males win aggressive encounters against larger conspecifics as much as 10–30% of the time (Small et al. 2009; Egge et al. 2011). Thus, taken alone, size does not entirely explain the outcome of a fight. Instead, a more detailed experimental approach combining analyses of differing fighting strategies with endogenous neurochemicals mediating such behaviors may be more consistent with actual outcomes.

The purpose of this study was to utilize the stalk-eyed fly as a model system to test the theoretical hypotheses of proposed contest models using a large previously collected dataset of intraspecific aggressive contests. Specifically, we aimed to determine whether simultaneously measuring behavioral, morphological, and neurophysiological variables could better explain contest outcome and structure than the simplified models using only morphological data. We show that although size plays an important role in winning a fight, it imperfectly predicts which males are victorious in size-mismatched contests. Interestingly, the data show a significant difference in fighting strategy between winners and losers, which appears largely independent of size. Rather, winners readily escalated encounters to higher intensity and physical contact and engaged in less LI, posturing behaviors compared with losers, no matter what the size disparity between opponents. To determine whether neurophysiological variables could account for these differing fighting strategies shown by winners and losers, we reanalyzed an additional dataset that contained size-matched opponents where half the males had pharmacologically elevated brain 5-HT. In doing so, we discovered that underlying discrepancies in brain 5-HT concentrations between winners and losers are important in directing expression of these differing fighting strategies. Moreover, the strategies associated with individuals possessing higher 5-HT than their opponents are reminiscent of those shown by winners in untreated contests. Therefore, factors such as biogenic amines should be considered in

future game-theory models for predicting individual expression of aggression and contest outcome.

Materials and Methods

Subjects

Teleopsis dalmanni is a sexually dimorphic species of stalk-eyed fly native to South East Asia. All laboratory-housed individuals are descendants of pupae obtained from the University of Maryland, College Park. Flies are reared communally in cages (45 cm × 22 cm × 19 cm) on a 12-h light:dark cycle with free access to food, water, and mating opportunities. Each cage is kept between 25–27 °C at ~80% humidity. All adult males used in the study were between 4–8 weeks post-occlusion. Eye span was measured to the nearest 0.01 mm using Scion Image (National Institutes of Health, Bethesda, USA) after brief anesthetization with CO_2 (Ribak and Swallow 2007). Eye span is highly correlated with body length, making it an accurate representation of body size (Burkhardt and de la Motte 1983; Wilkinson 1993). Individuals were given an identifying mark between their thoracic spines using an opaque paint pen and transferred to smaller cages (14 cm × 14 cm × 14 cm) containing ~10 individuals. Predetermined opponents were housed separately.

Forced-fight paradigm

All behavioral data presented were obtained from previously published studies (Egge et al. 2011; Egge and Swallow 2011) that used the same forced-fight paradigm, details of which can be found in Egge and Swallow (2011). Briefly, *T. dalmanni* were measured for eyestalk length to the nearest 0.01 mm and given an identifying mark between their thoracic spines. Twenty-four hours prior to the fight, flies were placed in a wood and glass arena, lined with moist filter paper. Opponents were kept separated by an opaque barrier, and no food was given. After 24 h of acclimation to the arena, the barrier was removed and a drop of corn media was presented in the center of the arena, which provided a contestable resource. Each fight was recorded by a digital video camera for 10 min. Behaviors were scored manually using JWatcher (Blumstein et al. 2007) with each fly's behavior scored independently of the other fly in the arena (i.e., each video was scored twice). Flies were assigned to be a winner or loser based on the number of retreat behaviors (turned away or quickly ran away) exhibited over the entire 10-min fight.

Drug administration studies

Data reanalyzed for investigation of the relationship of 5-HT and behavior were obtained from Bubak et al. (2014b). In all these studies, treated adult males ($n = 20$) were administered 3 g of the 5-HT precursor 5-hydroxy-L-tryptophan (5-HTP; H9772; Sigma, St. Louis, MO, USA) in 100 mL of food media containing pureed sweet corn, 25 mg of ascorbic acid, and 1 mL of methylparaben (Wilkinson 1993) as a mold inhibitor. Flies were fed *ad libitum* for 4 days. This treatment regime reliably elevates individual 5-HT concentrations in stalk-eyed fly brain tissue (Bubak et al. 2013). Opponents of treated flies ($n = 20$) were fed the same food media, sans 5-HTP, for 4 days.

Serotonin quantification

Serotonin (5-HT) in a single whole brain sample was detected by high-performance liquid chromatography with electrochemical detection as previously described (Bubak et al. 2013). Brain samples were frozen immediately after the fight, thawed, and centrifuged at 17,000 rpms. The supernatant was removed, and 45 µL of the sample was injected into the chromatographic system. The amines were separated with a C_{18} 4 -µm NOVA-PAK radial compression column (Waters Associates, Inc., Milford, MA, USA) and detected using an LC 4 potentiostat and a glassy carbon electrode (Bioanalytical Systems, West Lafayette, IN, USA). The sensitivity was set at either 0.5 or 1 n/V with an applied potential of +0.9 V vs. a Ag/AgCl reference electrode. The mobile phase initially was made by dissolving 8.6 g sodium acetate, 250 mg EDTA, 11 g citric acid, 330 mg octanylsulfonic acid, and 160 mL of methanol (all chemicals were obtained from Sigma-Aldrich, St. Louis, MO, USA) in 1 L of distilled water. In order to obtain the desired separation, additional increments of octanylsulfonic acid and methanol were added to the mobile phase. After removal of the supernatant for monoamine analysis, 60 µl of 0.4 M NaOH was added to the pellet to solubilize the remaining tissue for protein analysis (Bradford 1976). The CSW32 data program (DataApex Ltd., Prague, Czech Republic), set in internal standard mode, calculated 5-HT concentrations based on peak height values obtained from standards (all standards were obtained from Sigma-Aldrich). The resulting amine concentration was divided by microgram protein in the sample to yield pico gram amine/microgram protein after appropriate corrections for injection volume vs. preparation volume were carried out.

Statistical analysis

Separate unpaired Student *t*-tests were used to test for differences in the means of eyestalk length, flexing behaviors, HI (physical contact) to LI (non-physical) behavioral ratios, and 5-HT levels between winners and losers as well as larger and smaller opponents. To test for differences within and between groups in HI to LI behavioral ratios at different time points during the fight according to either status (winner/loser) or on body size relative to opponent, separate 2-way ANOVA (Analysis of Variance; time × either status or size) was applied, with Sidak's multiple comparison tests used to compare behavior between groups at each time point. Significant effects of time were followed by Sidak's tests to compare time point 1 against all subsequent time points to reveal differences within groups in HI/LI expression as the fight progressed. For these particular analyses, the same individuals did not contribute to data for every time point, precluding the use of a repeated measures ANOVA. All statistical tests were set at a 0.05 alpha level and conducted using Prism 6 (GraphPad software, La Jolla, USA).

Results

A total of 63 fights from previous size-matched and mismatched studies were quantified, with a distribution in size disparity between opponents ranging from 0% to 13% (measured by eyestalk length; Table 1). Winners were determined by fewest amounts of retreats relative to their opponent in a 10-min forced-fight paradigm.

Table 1. Range and mean of eyestalk length between winners and losers

	Size range (mm)	Mean size (mm ± SEM)
Winners (63)	7.25–8.75	8.03 ± 0.04
Losers (63)	7.23–8.66	7.86 ± 0.03
		$P < 0.001$ (Student's *t*-test)

Numbers of subjects contributing to these datasets are indicated in brackets. SEM: standard error of the mean.

Relationships with preexisting game theory models

Both models of self-determined persistence and mutual assessment predict a strong positive relationship with contest duration and loser RHP. Indeed, our data also support this prediction when eyestalk length is used as a measurement of RHP (linear regression; $R^2 = 0.064$; $P < 0.05$; $Y = 64.2x - 383.5$). However, our data did not indicate a significant relationship between contest duration and RHP difference between opponents (linear regression; $R^2 = 0.002$; $P = 0.72$; $Y = -10.9x + 126.3$), a prediction of both self-determined persistence and mutual assessment. Furthermore, our data did not suggest a significant relationship with winner RHP and contest duration (linear regression; $R^2 = 0.007$; $P = 0.51$; $Y = 18.7x - 26.6$), typically used as a key analysis to differentiate between both models. Therefore, our data do not seem to be consistent with either model when size is used as a proxy for RHP.

Relationships between fight strategy and body size

The fighting strategy of winners and losers differed in both LI (non-physical) and escalation patterns of aggressive behaviors. Losers engaged in significantly more flexing behaviors compared with their winning counterpart (unpaired *t*-test, $P < 0.05$, $df = 62$; Figure 1A). To test whether this posturing effect was a result of size relative to opponent, we analyzed flexing as a function of smaller and larger competitors, regardless of winning or losing, and found no significant difference between opponents (paired *t*-test, $P = 0.94$, $df = 61$; Figure 1B). There was also no significant difference between relative size differences and frequency of flexing behaviors (linear regression; $R^2 = 0.006$; $P = 0.57$; $Y = -1.51x + 10.77$). Together, this suggests that flexing is independent of relative size differences.

Next, we analyzed escalation patterns and total fight intensity between winners and losers by measuring the ratio of HI behaviors over LI behaviors expressed during the entire fight. The mean total time spent performing aggressive behaviors was approximately 120 s ± 6.1 s, no flies engaged in aggressive behaviors beyond 300 s. To examine how aggression was distributed across the 10-min forced-fight paradigm, we divided each contest into discrete time bins, 2-way ANOVA revealed effects of status ($F_{1,116} = 19.98$, $P < 0.001$), time ($F_{4,116} = 34.04$, $P < 0.001$) and an interaction between both factors ($F_{4,116} = 12.56$, $P < 0.001$). Subsequent pairwise comparisons showed that winners or losers engaging in aggressive behaviors for a total of 4 min or shorter (time points 1–4) in the 10-min fights did not differ in escalation patterns (Sidak $P > 0.05$; Figure 2A). However, when opponents engaged in aggressive behaviors for a total duration between 4–5 min (time point 5), winners performed significantly more HI than LI behaviors compared with losers in the latter stages of the fight (2-way ANOVA, $P < 0.001$, $df = 116$; Figure 2B). To test whether this was an effect of size, we ran the same analysis for larger and smaller opponents regardless of fight outcome and discovered that this difference in escalation pattern was abolished (2-way ANOVA, $P > 0.05$, $df = 116$; Figure 2B), suggesting that escalation patterns are largely independent of size. Additionally, there was no relationship between relative size difference and HI to LI behavior ratios (linear regression; $R^2 = 0.009$; $P = 0.46$; $Y = 0.25x + 0.44$). Although opponent size was not a reliable predictor of fighting strategy with respect to either escalation patterns or posturing, winners were significantly larger on average compared with losers (unpaired *t*-test, $P < 0.001$, $df = 124$; Table 1), suggesting that size still plays a crucial role in fight outcome.

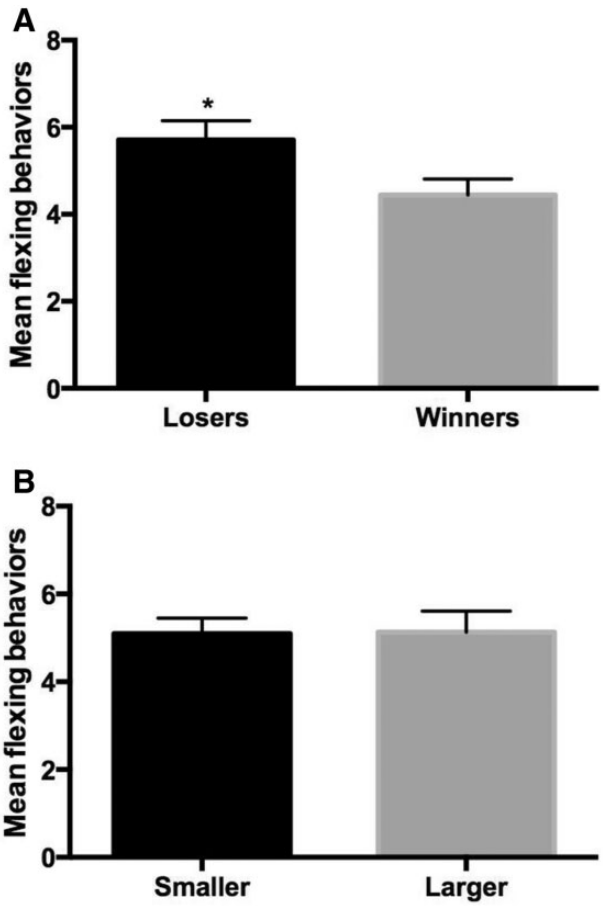


Figure 1. (A) When competitors were separated by status (winners and losers), losers performed significantly more flexing behaviors compared with winners (unpaired *t*-test, $P < 0.05$, df = 62). (B) Separation by size demonstrates no significant difference between flexing behaviors (unpaired *t*-test, $P = 0.94$, df = 61). Numbers presented as means ± *SEM*.

Relationships of fighting strategy with 5-HT

To investigate other potential factors besides size that influence fight intensity, we reanalyzed data from our previous studies where we had pharmacologically elevated neural 5-HT concentrations in half the male opponents in size-matched fights. Winners in these size-matched fights had significantly higher levels of 5-HT (unpaired *t*-test, $P < 0.01$, $df = 38$; Figure 3) as well as a greater HI to LI behavior ratio over the entire 10-min fight (unpaired *t*-test, $P < 0.05$, $df = 38$; Figure 3), indicating elevated intensity. In the absence of size differences, this mismatch in neural 5-HT concentrations appears sufficient to account for discrepancies in fight intensity between otherwise equal opponents. Thus, when taken together, neural 5-HT and size may represent a better predictor of a winner's fighting strategy than absolute or relative size alone.

Discussion

Stalk-eyed flies engage in both LI (non-physical) and HI (physical) aggressive behaviors during fights. Males follow a predictable, stereotyped escalation pattern starting with lining up of eyestalks, presumably for rival assessment, then progressively moving toward more intense behaviors (de la Motte and Burkhardt 1983; Egge et al. 2011; Bubak et al. 2014b). Similar to other animals, size seems to

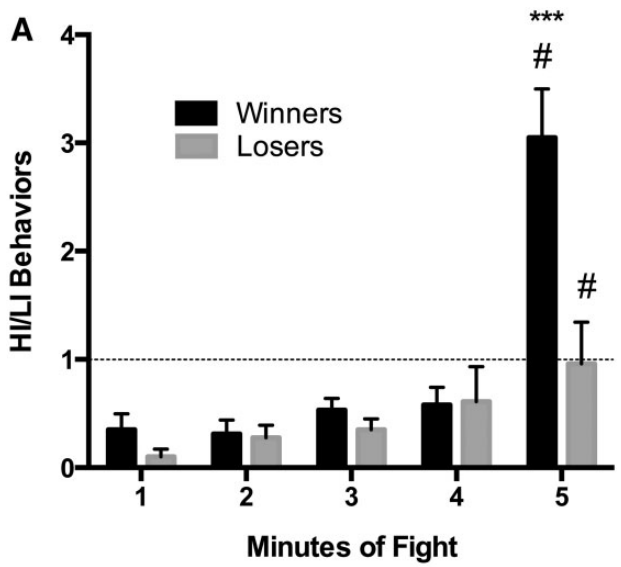


Figure 2. (A) Winners performed significantly more HI to LI behaviors compared with losers in fights that lasted between 4 and 5 min (time point 5). Both winners and losers had significantly higher HI/LI behavioral ratios at time point 5 compared with all other time points within their groups. (B) When separated by size, regardless of fight outcome, significantly higher HI/LI behavioral ratios are again seen at time point 5 compared with all other time points, but there is no difference between the groups. Asterisk (*) indicates differences between winners and losers at that time point; hash (#) indicates a significant within group difference compared with time point 1. Time points correlate with minutes spent fighting. Dashed line signifies a 1:1 ratio of HI behaviors to LI behaviors.

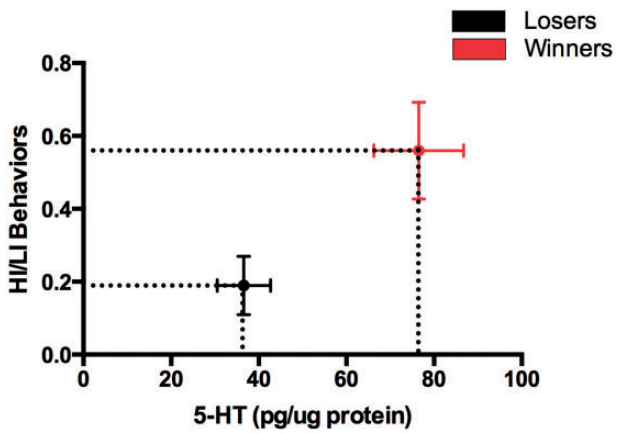


Figure 3. In size-matched fights, winners had significantly higher brain 5-HT levels (x axis; unpaired t-test, $P < 0.01$, df = 38; mean ± SEM) as well as a higher HI/LI behavioral ratio (y axis; unpaired t-test, $P < 0.05$, df = 38; mean ± SEM) compared with losers.

play an important role in the outcome of these aggressive encounters, with the larger male typically succeeding in routing his opponent. However, when taken alone, size cannot completely predict specific winning and losing strategies in this species, and should be but 1 factor in determining fighting ability of individual animals. This is demonstrated by our reanalysis of data obtained from previous studies investigating the relationship between central monoamine levels and aggressive motivation and fighting strategy in size-matched contests. Therefore, we propose that future models attempting to measure assessment strategy (e.g., self-determined persistence vs. mutual rival assessment) and fight outcome based on individual RHP should incorporate neurophysiological information.

We hypothesized that losers engaged in significantly more flexing behaviors compared with winners as a technique for smaller males to "bluff" their opponent without actually engaging in physical contact behaviors. Use of such a strategy would be in accordance with mutual rival assessment theory, with smaller males exhibiting less intense aggression after perceiving a larger opponent. However, we saw no difference in flexing between smaller vs. larger opponents, with losing males always displaying more flexing. This suggests that individual males employing this ultimately losing strategy do so regardless of size discrepancies. In contrast, winners engaged in more HI, physical contact behaviors, but this only became evident if the fight lasted more than 4 min. Again, this was independent of opponent size. The retention of LI behaviors by losers despite body size differences argues against predictions made by either the mutual rival assessment or self-determined persistence models when using size as the sole measure of RHP (e.g., Arnott and Elwood 2009). Similarly, the fact that size-independent differences in aggressive behavior by winning males only appeared after the contest had exceeded a certain duration suggests factors other than physical indicators of RHP are modulating individual responses to social challenge in this species.

To understand what other factors may be influencing these differing escalation patterns, we reanalyzed a dataset of size-matched males with half the opponents containing pharmacologically exaggerated endogenous levels of brain 5-HT. In doing so, we discovered that elevated brain concentrations of 5-HT were sufficient to mimic the winning strategy of the randomly paired opponents. Specifically, treated males with higher 5-HT but identical in size to their opponent engaged in significantly more HI behaviors than LI behaviors. This is remarkably similar to the pattern shown by winning opponents in contests of varying size disparity, and suggests that endogenous brain levels of 5-HT, and possibly other biogenic amines such as OA, may be responsible for the escalation patterns shown by winners during longer duration fights. We have previously demonstrated that raising 5-HT in a smaller competitor will lead to increased willingness to engage in HI encounters with larger opponents in stalk-eyed fly males (Bubak et al. 2015). However, smaller

opponents treated with 5-HT were not more likely to win the fight compared with their control counterpart, because the larger opponent preemptively escalated the contest to physical contact behaviors. This suggests that the larger opponent, when faced with a treated, hyper-aggressive smaller opponent, switches his escalation pattern after gathering specific information about its rival's aggressive state. This may be an explanation for the sudden switch in escalation patterns seen in this study. Taken in this respect, applying 5-HT as the predicting variable to existing theoretical models may produce different outcomes for winners and losers depending on the model employed. For instance, our data suggest that losers trend toward fitting the self-determined persistence model, with aggression increasing as 5-HT levels approach those of the opponent. In contrast, 5-HT only predicts HI aggression as the fight continues and the winners have had the opportunity to perceive the opponent's aggression, which is more in agreement with predictions of the mutual rival assessment model.

Perception of size for opponent assessment is still clearly playing a role in contest outcome, as the majority of winning males were larger than their opponents. Size can predict escalation and intensity patterns within the first 4 min of a fight, so it may be possible that initial perception of size is the primary determining factor for short-lived encounters in stalk-eyed flies, especially for opponents that go on to lose the contest. However, it does not explain why winners would only escalate intensity as fights progressively become more costly and time-consuming. Although possessing higher 5-HT may partly account for this change in behavioral expression, as discussed above, perception of other cues may also contribute to the motivation to stay in the contest or escalate physically, usurping size as the determining factor in fight outcome. This possibility is suggested by studies showing that the fighting strategy of smaller crickets changes when visual perception of the opponent is not possible, with blinded crickets fighting for longer and at higher intensity against a larger opponent with disabled mandibles that could not inflict physical damage (Rillich et al. 2007). Given that smaller crickets will normally flee from a larger rival in the opening stages of an interaction (Rillich et al. 2007), this implies that opponent assessment and potential risk of engagement is based on a combination of both size perception and damage accrual as the contest progresses. We found that winning flies exhibited less flexing and more HI behaviors than losers, even when opponents were matched in size, suggesting similar cumulative perception of cues and risk assessment as the fight continued. Specifically, eventual winners may be gathering information throughout the fight about their opponent in addition to perception of size alone, possibly from the excess of posturing (flexing) behaviors performed by the losers. This collated information then conveys a lower threat level to winners, promoting the confidence to escalate the fight. Consistent with this idea, fights shorter than the 4-min mark may not provide sufficient time to gather necessary information about the opponent, resulting in both winners and losers engaging in a similar ratio of HI to LI behaviors.

In summary, we have described 2 different fighting strategies between winning and losing male stalk-eyed flies. Additionally, we have found that these strategies are largely independent of relative body size, one of the most commonly used proxies of RHP for game theory modeling and assessment strategies. Brain levels of 5-HT appear to provide ability to predict which individuals will display less intense aggression and go on to lose the fight, whereas a combination of size, opponent assessment and higher 5-HT could explain the escalation pattern of the winning fighting strategies. The contribution of endogenous 5-HT to both fighting strategies suggests that it is imperative that future game theory and assessment models include neurochemical factors when attempting to decipher the mechanisms of animal contests.

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