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ORIGINAL PAPER

Sequential analysis of aggressive interactions in the stalk-eyed fly *Teleopsis dalmanni*

Alison R. Egge · Yoni Brandt · John G. Swallow

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Abstract Understanding the mechanisms and determinants of conflict resolution is of great theoretical and practical importance because the outcome of contests between males over limited resources such as mates, territories, and food has profound fitness consequences. Despite the large literature on the theory of conflict resolution, relatively few empirical studies explicitly test predictions related to contest structure for these models. In sexually dimorphic species of stalk-eyed flies (Diopsidae), males engage in characteristic aggressive interactions over both females and food resources. We used sequential analysis of aggressive interactions between dyads of male stalk-eyed flies to investigate patterns of escalation, behavioral matching, and physical contact in order to distinguish between three common models of conflict resolution: the sequential assessment model, the cumulative assessment model, and the energetic war of attrition. Stalk-eyed flies were shown to engage in both low- and high-intensity behaviors during interactions with patterns of escalation and no de-escalation. Aggressive interactions did not demonstrate behavioral matching between winners and losers. Stalk-eyed flies also escalated to behaviors that included physical contact without injuries. Our results provide support for the sequential assessment model based on patterns of escalation with no de-escalation, behavioral mismatching, and behaviors which include physical contact but no injuries.

Keywords Conflict resolution · Assessment · Aggression · Stalk-eyed fly · Sequential analysis

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A. R. Egge · Y. Brandt · J. G. Swallow (✉)
Department of Biology, The University of South Dakota,
414 E Clark St, Churchill-Haines 179,
Vermillion, SD 57069, USA
e-mail: John.Swallow@usd.edu

Introduction

In many animal species, individuals fight over access to resources, such as mates and food (Huntingford and Turner 1987). Ritualized activities and specialized structures are often used during these aggressive encounters (Emlen 2008; Geist 1966), but there is still a great deal of debate about the precise role these activities play in determining the course and outcome of animal contests (Briffa and Elwood 2009). Because fighting can be costly, many animals employ morphological ornaments, presumably to gauge their opponents' fighting abilities in order to avoid additional costs (Enquist 1985; Small et al. 2009). Individuals use mutual assessment to compare asymmetries in ornament size to determine asymmetries in fighting ability and thus how to proceed with a contest (Enquist and Leimar 1983; Panhuis and Wilkinson 1999). Recently, the possibility that animals utilize these assessment behaviors and structures as signals for the mutual assessment of one another's capabilities has been contrasted with the alternative that contest outcome is determined by the eventual loser's internal persistence threshold (i.e., "self-determined persistence"; Taylor and Elwood 2003). In both the mutual assessment model and the self-determined persistence model, larger individuals usually win a contest. When size asymmetry is low or information on sizes is lacking, additional behavioral data are needed to distinguish between the two models (Taylor and Elwood 2003). These

behavioral analyses of escalation patterns of aggressive behavior, behavioral matching, and physical contact can then be applied to three subsets of the mutual assessment hypothesis and the self-determined persistence hypothesis in order to fully discriminate between the two general models of conflict resolution. These three-game theory models are the sequential assessment model, the cumulative assessment model, and the energetic war of attrition.

In mutual assessment, both individuals perform agonistic behaviors that provide reliable information to their opponent, allowing assessment of relative strength, with the weaker rival conceding the resource without incurring the full cost of a fight (Gammell and Hardy 2003; Taylor and Elwood 2003). This type of assessment is captured in the sequential assessment model (SAM), where rivals attempt to ascertain which contestant is stronger, by performing increasingly costly behaviors and thereby progressively acquiring further accurate information as the interaction progresses and escalates (Enquist and Leimar 1983; Enquist et al. 1990). The SAM predicts that contests progress through gradually escalating phases where individuals can reach costly physical contact but direct injury to opponents is rarely observed and no de-escalation of aggressive behavior occurs (Briffa and Elwood 2000; Enquist and Leimar 1983; Enquist et al. 1990; Kelly 2006). However, behavioral matching, meaning non-differing frequencies of behaviors between winners and losers, between rivals is not consistent under this model (Briffa and Elwood 2000, 2009; Payne 1998). The SAM also predicts that contest duration should be negatively correlated with asymmetry in resource holding potential (RHP) (Enquist et al. 1990) and that loser RHP should be positively correlated with contest duration while winner RHP will be negatively correlated with contest duration in SAM (Arnott and Elwood 2009). Red deer stags and *Drosophila* have both been shown to follow SAM during conflict resolution (Chen et al. 2002; Clutton-Brock et al. 1979).

In the self-determined persistence hypothesis, the decision to retreat is determined by the loser's innate threshold for injuries sustained or costs accrued and not assessment of the rival's fighting ability gathered during the interaction (Dietemann et al. 2008; Taylor and Elwood 2003). This provides an alternative model to mutual assessment for contest resolution. Instead of assessing rivals to determine escalation and when to retreat based on perceived rival strength (Gammell and Hardy 2003; Morrell et al. 2004), individuals use internal cues to determine whether to fight or flee. In the energetic war of attrition (E-WOA), a self-determined persistence model (Mesterton-Gibbons et al. 1996; Payne and Pagel 1996), escalation may rise, be maintained, or decline at any time during a contest (Briffa and Elwood 2009; Gammell and Hardy 2003; Taylor and Elwood 2003). Individuals engage in displays of endurance, and no physical contact between opponents occurs (Kelly 2006; Mesterton-Gibbons et al. 1996). Additionally, opponents match behavioral tactics throughout most of the contest and thus are expected to show similarities in behavioral transitions (Briffa and Elwood 2009). The decision to retreat is made when the loser reaches its internal threshold. Contest duration is then predicted to be positively correlated with the loser's size (or RHP) as well as follow a shallow, positive correlation with winner size (Arnott and Elwood 2009; Mesterton-Gibbons et al. 1996; Taylor and Elwood 2003). Damselflies and the spider *Argyrodes antipodiana* both follow the predictions presented by E-WOA during conflict resolution (Marden and Waage 1989; Whitehouse 1997).

A third model which incorporates aspects of both SAM and E-WOA is the cumulative assessment model (CAM; Payne 1998). As with SAM, an individual's behavior is influenced by their rival, since stronger individuals inflict costs at a higher rate, but no assessment occurs and the decision to retreat is made according to individual internal thresholds and the costs accrued through physical contact (Briffa 2008; Payne 1998). Additionally, as in E-WOA, escalation can increase, decrease, or be maintained within the contest (Briffa and Elwood 2009; Taylor and Elwood 2003). Conversely, CAM also predicts that winners and losers will not show patterns of behavioral matching (Briffa and Elwood 2009; Taylor and Elwood 2003), as winners and losers tend to escalate at different rates during a contest (Payne 1998). Individuals can be subjected to physical contact and potential damage from their opponents (Kelly 2006; Payne 1998). The CAM also predicts that contest duration will increase as the loser's size (or RHP) increases, but it will also be influenced by the opponent's ability to inflict injuries. Hence, as in SAM, contest duration is expected to increase as rival size disparities decline (Payne 1998) as well as demonstrate a positive correlation between loser RHP and contest duration and a negative correlation between winner RHP and contest duration (Arnott and Elwood 2009). The Wellington tree weta has been shown to follow predictions of CAM in its conflict resolution (Kelly 2006).

Male stalk-eyed flies (Diptera: Diopsidae) exhibit morphology and behaviors that appear to facilitate mutual rival assessment in their daily contests for feeding territories and harems of reproductive females at nighttime roosts. The eyes of stalk-eyed flies are positioned at the tips of elongated cephalic appendages, and the length of these eyes talks is highly correlated with body size (Burkhardt and de la Motte 1983; Wilkinson and Dodson 1997). Males confront their rivals head-on with their eye stalks aligned in parallel, presumably affording each individual a precise measure of the relative size of its opponent (de la Motte and Burkhardt 1983; Panhuis and Wilkinson 1999). During

lengthy encounters, males may engage in front leg batting or tussling in order to remove the rival male and procure a territory (Burkhardt and de la Motte 1983; Wilkinson and Dodson 1997). Since prolonged interactions can impose costs such as lost time foraging or mating, energy loss, and injury, the use of eye stalks as reliable indicators of rival size and strength would be evolutionarily advantageous to minimize costs (Wilkinson and Dodson 1997).

Panhuis and Wilkinson (1999) found that as the disparity of eye span decreased mean contest duration increased in stalk-eyed flies *Cyrtodiopsis dalmanni* and concluded that these results support the mutual assessment hypothesis. However, their analysis was not specifically designed to distinguish between mutual assessment and self-determined persistence. A re-analysis of their data showed a negative correlation between loser size and contest duration and nonsignificant, positive correlation between winner size and contest duration, consistent with self-determined persistence (Brandt and Swallow 2009; Taylor and Elwood 2003). [The genus *Cyrtodiopsis* has recently been synonymized with *Teleopsis* (Meier and Baker 2002), and we will be using the updated nomenclature from here on.] Another study of stalk-eyed flies indicated that pairs of large males fight for a longer duration than pairs of small males or large–small pairs (Small et al. 2009), a result which suggests that smaller males have lower internal thresholds during interactions and is consistent with the self-determined persistence hypothesis. However, because in mutual assessment, a rival's RHP is assessed and compared explicitly to self, there must be a component of self assessment; this result is not completely inconsistent with mutual assessment (Enquist et al. 1990; Enquist and Leimar 1983). Although these results, taken together, suggest mutual rival assessment may play a smaller role than previously anticipated in this system, the assessment mechanisms employed by stalk-eyed flies remain unclear (Brandt and Swallow 2009) and are at this point equivocal between mutual assessment and self-determined persistence.

To differentiate between the assessment mechanisms used by Diopsid flies, our study employs an analysis of the sequence of male aggressive behaviors during dyadic interactions. Organizing aggressive behaviors in a time-sensitive form provides a quantitative description of the dependencies of each behavior (Castellan 1979; Gottman and Roy 1990) and provides information on patterns of escalation, including whether and when there is physical contact between opponents. Moreover, sequential analysis yields measures of behavioral transitions frequencies, which can be compared between winners and losers to test for behavioral matching between opponents. The SAM, CAM, and E-WOA each predict a unique combination of these three characteristics (escalation, behavioral matching, and physical contact) and sequential analysis can provide the necessary data to accurately differentiate between these models (Chen et al. 2002; Jennings et al. 2005; Table 1).

Methods

Subjects

We studied *Teleopsis dalmanni*, a stalk-eyed fly species native to the tropics of Asia (Burkhardt and de la Motte 1983; de la Motte and Burkhardt 1983). The current captive-bred populations of stalk-eyed flies were obtained from Gerald Wilkinson (University of Maryland—College Park). Flies in the laboratory were raised in plastic cages (45×22×19 cm) at 80% humidity and 23–25°C on a 12-h light/dark cycle (Wilkinson 1993). Flies were raised in cohorts of 100 and were maintained on ground corn medium and supplemented with Ward's *Drosophila* food. Flies were allowed ad libitum access to females and water, and cages were furnished with strings that served as mating areas, simulating rootlets in nature (Burkhardt and de la Motte 1983). To control for the effects of age, males used in our experiments were sexually mature, at least 3 weeks post-occlusion but less than 2 months post-occlusion (Baker et al. 2003).

Contest protocol

Two male stalk-eyed flies were randomly selected from different population cages without regard to eye-span length, anesthetized using CO_2, and painted with a drop of quick drying paint (DecoColor) on their thorax. Each was given a number and color identification (blue, green, red, or white). Each fly was placed lying on its thoracic spines under a dissecting microscope at X15-20 magnification and photographed using a digital camera. Subjects were then placed in an arena (11×6.5×5 cm) lined with moist filter paper and separated by a removable barrier. The arena consisted of three wooden walls painted white, a forward facing glass wall for increased lighting, and a removable glass top for introducing flies and through which video recording of interactions took place. The two flies were then starved for 24 h and allowed to acclimate to the arena in the same climatic conditions in which they were raised. After 24 h, a drop of corn medium (approximately 4 mm in diameter) was placed in the center front of the arena using a sterile syringe needle and the partition was removed. The interaction was then digitally recorded for 10 min. Over the course of each 10-min trial, dyads interacted in multiple contests. Each fly was only used for one interaction to prevent winner/loser effects from confounding the results.

Table 1 Summary of differences in contest dynamics predicted by three-game theory models of contest resolution

Game theory model	Patterns of escalation	Behavioral matching	Duration dependence	Physical contact	References
Sequential assessment	Escalation only	No	Positive correlation to loser RHP Negative correlation to winner RHP	Yes, no injuries	Enquist and Leimar 1983; Enquist et al. 1990
Cumulative assessment	Escalation with de-escalation	No	Positive correlation to loser RHP Negative correlation to winner RHP	Yes, injuries possible	Payne 1998
Energetic war of attrition	Escalation with de-escalation	Yes	Positive correlation to loser RHP Shallow, positive correlation to winner RHP	No	Mesterton-Gibbons et al. 1996; Payne and Pagel 1996

Our results indicate that there was escalation of aggressive behaviors with no de-escalation, there was no behavioral matching, and there was physical contact but with no detectable injuries

Ethogram

To determine the behavioral repertoire of male stalk-eyed flies, ten trials were haphazardly chosen and scored; observed behaviors were then integrated into a comprehensive ethogram. The start of each aggressive contest was defined by either the parallel lining up of eye stalks or an individual's approach of their opponent, and the end of each contest was determined when the flies were greater than one body length apart or not directly facing each other for three or more seconds (Table 2). Behaviors that were included in the ethogram were those that occurred solely during aggressive interactions when opponents were engaged with one another. Actions that occurred between interactions were not included in the analysis. Low-intensity behaviors were those in which no physical contact occurred between opponents. High-intensity behaviors were those that involved physical contact between opponents. Conflict resolution behaviors were defined as those behaviors that could potentially indicate the contest conclusion (i.e., pursuit, retreat, and moving away from opponent). Each behavior was mutually exclusive and exhaustive, meaning only one behavior could occur and be recorded at any given time, and all behaviors observed were accounted for in the ethogram.

Analysis

We scored all interactions with JWatcher, a free behavior analysis program (Blumstein et al. 2007). The behavior of each fly was scored independently of its opponent so that each video was scored twice, once for the winner and once for the loser. A fly was determined to be a loser if it turned away to retreat or if it quickly ran away from its paired conspecific more often than the rival fly over the course of the 10-min interaction (Panhuis and Wilkinson 1999). Conversely, the fly that showed fewer retreat behaviors than its rival was scored as the winner. Multiple contests occurred within each individual trial beginning when one fly approached another or the pair lined up eye stalks in parallel and ending when the flies were at least one body length apart or not engaged with one another for 3 s. Winners and losers were also established for each individual contest based on retreat and pursuit behaviors. Trial winner/loser classification was determined based on the sum of retreat behaviors from all contests occurring within the 10-min interaction.

Sequential analysis, relying on single-order Markov chains, was used to test for the existence of stereotyped temporal structure in behavior patterns that occur non-randomly. Sequential analysis produces observed behavioral matrices and transitional probability matrices that allow us to establish whether transitions from preceding behaviors to subsequent behaviors are significantly different than expected if behaviors do not proceed in a stereotypical order. The observed matrix provides the occurrence of one behavior following another behavior as a count of each behavioral transition during the interaction. Transitions refer to the relationship between two exclusive behaviors; for example, the transition of line up eye stalks → flex/extend refers to the antecedent behavior of lining up of the eye stalks followed by flexing of the forelegs. The transitional probability matrix provides the frequency of each transition. Standardized z scores and p values were used to determine the significance of the transitions in the transitional probability matrix in order to limit our analysis to only significant transitions within the interactions (Blumstein and Daniel 2007). All z scores ±1.96 standard

Table 2 Ethogram of aggressive behaviors observed among dyads of male *T. dalmanni*

Behavior	Description
Low-intensity behaviors	
Approach opponent	Movement toward opponent
Line up eye stalks	Oriented with eye stalks to opponent's in parallel fashion
Rear up	Uses mid- and hind-legs to rise in posture
Flex and extend forelegs	Bends and flicks forelegs near own eye stalks; directed at opponent
High-intensity behaviors	
Jump attack	Jumps on opponent's dorsal side
Attack/lunge	Uses forelegs in downward motion, striking at or on opponent
Tussle	Entangled with opponent, either clasping opponent's forelegs or eye stalks
Conflict resolution behaviors	
Away	Turning away or slowly moving away from opponent
Pursue	Quickly moving after opponent while no longer facing opponent
Retreat	Quickly moving from opponent while no longer facing opponent
End	Eye stalks are no longer aligned for a minimum of 3 s or flies are at least one body length apart

deviations were used to determine significance of transitions at the 0.05 probability level (Gottman and Bakeman 1986). In a previous study, uncorrected z scores at a 0.05 α level provided a powerful estimate of significance when used with multiple comparisons and in only two cases were z scores are not a reliable indicator of significance, both of which had low sample sizes (Bakeman and Gottman 1997). Therefore, inflated type 1 error due to uncorrected z scores should not be of concern when sample sizes are adequate as in our study (Slooten 1994). However, we performed a Bonferroni correction for sample size of 18, which gave us a corrected $\alpha<0.0027$.

Interactions in which flies did not directly compete with each other (14 out of 106 trials) were not included in the analysis. We conducted an analysis of aggressive behaviors for the stalk-eyed flies by pooling the interactions of all flies to produce and quantify an overall pattern of aggressive behaviors and to determine whether or not escalation of aggressive behaviors was observed. Transitions consistent with escalation are those from a low intensity, non-contact behavior to a high-intensity behavior that includes contact; similarly, for de-escalation, the transition would be from a high-intensity behavior to a low-intensity behavior. Transitions to conflict resolution behaviors are not included as de-escalation since conflict resolution behaviors are not classified as low intensity or high intensity. We used the behavioral transition matrix to construct kinematic diagrams of behavioral transitions (Chen et al. 2002), which visually depict the frequency of each behavior, frequency of transitions between behaviors, and the transition of one behavior to another. Statistically significant transitions that occurred greater than 10% of the time throughout an interaction were included in the kinematic diagrams.

To determine whether the opponents differed with regard to behavioral transitions, the behavioral transition matrices of trial winners were compared to those of trial losers using a Pearson's chi-square test of independence using SAS version 9.1 (SAS Institute Inc. 2002–2004). After comparing winners and losers with all behaviors included, we re-analyzed the data with pursuit and retreat excluded from the dataset. These two behaviors were the defining determinants of winners and losers and could confound the chi-squared analysis. This analysis provides information on behavioral matching between winners and losers by excluding the variables that we used to define winners and losers. We also contrasted behavioral transition matrices of contest winners and losers. Transitions that occurred more or less frequently in losers than in winners than expected exhibited high chi-squared values (significant values, chi-squared>37.65, df=25; chi-squared>33.92, df=22; chi-squared>26.30, df=16; chi-squared>18.31, df=10).

Results

We observed a total of 567 contests in 92 trials with an average of 6.1±4.8 (mean±standard deviation) contests per trial (range 1–17). On average, contests lasted for 9.0±0.01 s (range 1 s–1 min 29 s). Lining up of eye stalks occurred the most often of any other behavior (32% of all behaviors), followed by flexing of the front legs (15%) and rearing up (10%). The two behaviors that occurred least often were tussling (3.5%) and jump attack (1.4%). Trials reached high-intensity behaviors at least once 48 of 92 times (52%), and 132 out of 567 contests (23%) reached high-intensity behaviors, indicating that one pair is not significantly driving results in one direction.

Several behavioral transitions occurred more frequently than expected by chance in the behavioral analysis of aggressive interactions in stalk-eyed flies (Table 3; Online Resource 1). Transitions were grouped into low–low intensity transitions, low–high intensity transitions, high–high intensity transitions, conflict resolution–low intensity, and lastly transitions including end-of-contest behaviors. There were no significant high–low transitions during overall trials (i.e., from behaviors of physical contact to those without physical contact, not including conflict resolution behaviors), which would be expected if de-escalation were occurring. Low–low intensity transitions that occur most frequently included approach opponent → line up eye stalks and line up eye stalks → flex/extend legs. Tussle → attack/lunge is a high–high transition that occurred with 49% frequency in transition. Transitions that occurred most frequently that included conflict resolution behaviors are retreat → end contest, pursuit → end contest, and away → end contest. Significant behavioral transitions and their frequencies are shown in a kinematic diagram for the overall behavioral transitions of aggressive interactions in stalk-eyed flies (Fig. 1).

Table 3 Transitional frequencies that occurred more frequently than expected by chance in the contests of male *T. dalmanni*

Behavioral transition	Frequency of transition (%)
Low intensity to low intensity	
Approach → lining up eye stalks	84
Line up eye stalks → flex	42
Line up eye stalks → rear up	23
Flex → rear up	15
Low intensity to high intensity	
Rear up → attack	26
High intensity to high intensity	
Tussle → attack	49
Attack → tussle	38
Conflict resolution to low intensity	
Away → line up eye stalks	29
Pursue → line up eye stalks	29
Conflict resolution	
Retreat → end	83
Pursue → end	66
Away → end	59
Jump attack → pursue	49
Flex → away	21
Attack → pursue	16
Rear up → pursue	14
Tussle → end	12
Attack → away	*11*
Rear up → away	*10*

All transitions are significant at an uncorrected $\alpha<0.05$; italics indicates transitions that are not significant with Bonferroni-adjusted $\alpha<0.0027$

The behavioral transition frequencies of trial winners differed significantly from those of trial losers ($\chi_{25}^2=416.56$, $p<0.0001$; individual behavioral transitions that differed are listed in Table 4). Only two behavioral transitions differed significantly for winners and losers including retreat → end and pursuit → end (Table 4). A kinematic diagram of trial winner and loser transitions and their frequencies highlights the behavioral transitions during aggressive interactions (Fig. 2). Behavioral transitions differed significantly when pursuit and retreat behaviors were removed from chi-squared analysis as well ($\chi_{16}^2=56.85$, $p=0.0001$).

The behavioral transition frequencies of contest winners differed significantly from those of contest losers ($\chi_{22}^2=837.35$, $p<0.0001$; individual behavioral transitions that differed are listed in Table 5). Three behavioral transitions differed significantly for contest winners and losers including retreat → end, pursuit → end, and flex/extend legs → retreat (Table 5). A kinematic diagram of contest winner and loser transitions and their frequencies show the significant behavior transitions (Fig. 3). Chi-squared analysis with removal of pursuit and retreat behaviors also differed significantly for contest winners and losers ($\chi_{10}^2=22$, $p=0.0001$). Results were qualitatively similar regardless of whether the analyses were based on entire trials or individual contests, so references to winners and losers hereafter refer to both trial and contest data.

Discussion

We set out to quantitatively describe the sequence of aggressive behaviors in male stalk-eyed flies during staged interactions over a food resource to infer patterns of escalation, behavioral matching, and physical contact to distinguish between several common models of contest resolution. Our general analysis, which included both winners and losers, revealed a significant degree of behavioral structure, including evidence of aggressive escalation. When looking across all flies, we found a general pattern in which flies transitioned from low-intensity, no-contact behaviors to high-intensity behaviors which included physical contact (Fig. 1). Low-intensity behaviors such as lining up of eye stalks, flexing, and rearing up occurred the more frequently than any other behaviors, suggesting much time is spent avoiding potentially costly high-intensity behaviors. These patterns suggest that stalk-eyed flies are successfully using these ritualistic, low-intensity behaviors to avoid physical contact by subsequently transitioning to conflict resolution rather than initiating a fight (Briffa 2008; Briffa and Elwood 2009). The low-intensity behavioral transition of approaching an opponent to lining up eye stalks

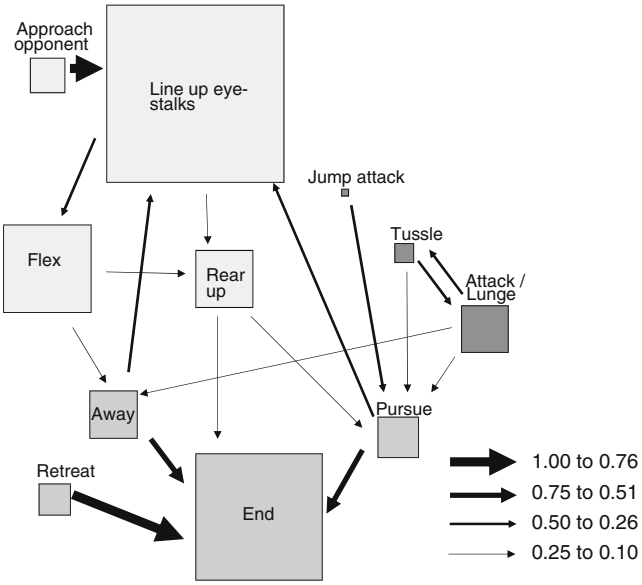


Fig. 1 Kinematic diagram of behavioral transitions that occurred more frequently than expected by chance in contests of male *T. dalmanni*. All transitions occurred greater than 10% of the time and are significant at $p<0.050$. *Box size* indicates frequency of behavior; *arrow thickness* indicates probability of occurrence. *Light shading* is indicative of low-intensity behaviors, *dark shading* indicates high-intensity behaviors, and *medium shading* indicates end-of-contest behaviors

occurred at a very high frequency (84%), suggesting that — almost invariably— if one opponent approaches another, a contest will occur.

Analyses of behavioral differences between winners and losers provide no evidence for behavioral matching (Tables 4 and 5). Low-intensity behaviors occurred at high frequencies for both winners and losers and patterns of escalation were evident in both winners and losers, but escalation occurred at significantly different transition frequencies (Figs. 2 and 3). For example, trial losers significantly transitioned from lining up eye stalks → flex more than trial winners. High-intensity behaviors occurred less frequently than low-intensity behaviors, but did occur in both winners and losers, showing that stalk-eyed flies are exhibiting escalation, including behaviors with physical contact and no detectable injuries. Our first analysis of the differences between winners and losers, which included pursuit and retreat behaviors, suggested that behavioral frequencies were matched up until the point of conflict resolution (Fig. 2). However, when pursuit and retreat behaviors were removed to control for consequences of scoring, winners and losers still exhibited significantly different behavioral transitions.

Even though escalation of behaviors is predicted for SAM, CAM, and E-WOA, the patterns of escalation differ dramatically between models. In SAM, escalation of aggressive behaviors occurs between phases and no de-escalation is predicted (Briffa and Elwood 2000, 2009). The E-WOA and CAM predict that escalation can occur with rises, falls, and maintenance of intense behaviors occurring throughout the contest (Mesterton-Gibbons et al. 1996). When examining overall patterns of aggression in stalk-eyed flies, escalation occurs with a ritualistic pattern, flowing from low-intensity behaviors to high-intensity behaviors throughout an interaction (Fig. 1). Time spent in low-intensity behaviors is also higher than time spent in high-intensity behaviors, conforming to the avoidance of high-intensity costly behaviors unless necessary, a key assumption in the mutual assessment hypothesis (or SAM) (Enquist and Leimar 1983; Panhuis and Wilkinson 1999). In both winners and losers, high-intensity behaviors occur after escalation from low-intensity behaviors without de-escalation (Figs. 1, 2, and 3), showing that stalk-eyed flies are engaging in ritualized, escalated agonistic interactions, providing support for SAM (Briffa and Elwood 2009; Enquist and Leimar 1983; Taylor and Elwood 2003). Our overall sequential analysis, which includes both winners and losers, does not include any transitions that are consistent with de-escalation but does reveal a pattern of behavioral transitions that indicate the flies will only escalate to increasingly intense behaviors (Table 3, Fig. 1). When separated into winner and loser behaviors, one can see patterns of re-orientation but not a step-down decrease consistent with de-escalation. For example, in losers, we see a minor frequency of transition from jump attack → lining up eye stalks (Figs. 2 and 3). However, this pattern does not

Table 4 List of behavioral transitions that contributed significantly to the high chi-squared difference for trial winners and losers with pursue and retreat behaviors ($\chi_{25}^2=416.56$, $p<0.0001$) and without pursue and retreat behaviors ($\chi_{16}^2=56.85$, $p<0.0001$)

Behavioral transition	Chi-squared value	Winner transition (%)	Loser transition (%)
Retreat → end contest	60	71	85
Pursue → end contest	44	70	54
Away → line up eye stalks[a]	4.13	26	36
Flex → away[a]	3.85	18	23
Line up eye stalks → flex[a]	4.16	35	46

[a] Transitions without pursue and retreat behaviors in analysis

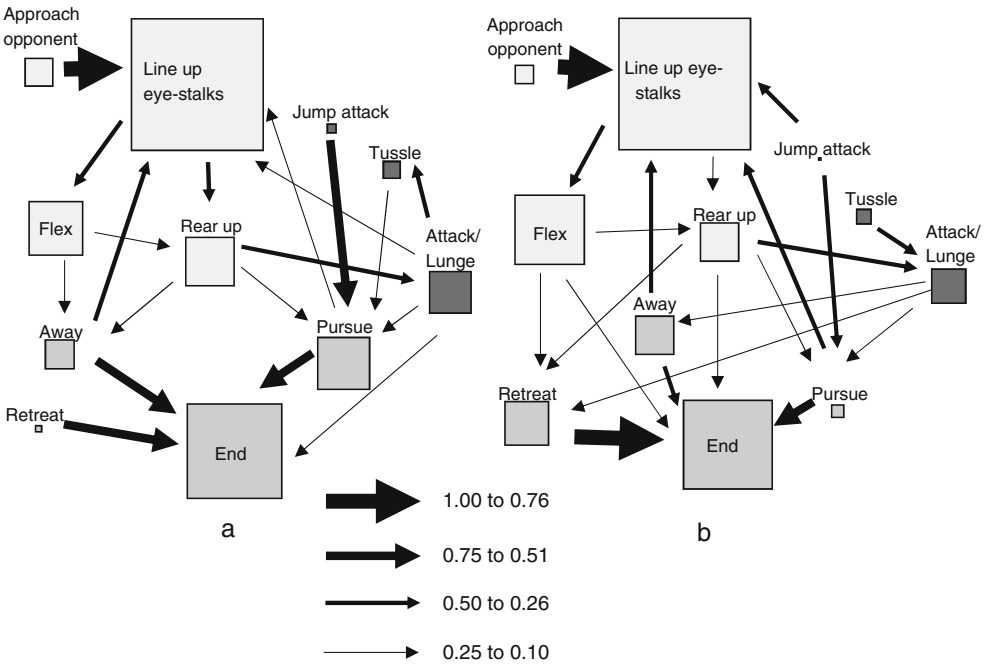


Fig. 2 Kinematic diagram of trial winner (**a**) and loser (**b**) behavioral transitions that occurred more frequently than expected by chance in contests of male *T. dalmanni*. All transitions occurred greater than 10% of the time and are significant at $p<0.050$. *Box size* indicates frequency of behavior; *arrow thickness* indicates probability. *Light shading* is indicative of low-intensity behaviors, *dark shading* indicates high-intensity behaviors, and *medium shading* indicates end-of-contest behaviors

indicate de-escalation because the jump attack behavior is often used for losers as a surprise attack behavior; during this behavior, one opponent will jump onto their rival, after which they will immediately line up their eye stalks in parallel to reorient themselves to potentially engage in another contest. This pattern of escalation with no de-escalation is consistent with SAM but is inconsistent with both CAM and E-WOA (Arnott and Elwood 2009).

Physical contact and injuries are also instrumental in differentiating the game theory models from one another. Analysis of the aggressive interactions between stalk-eyed flies shows that behaviors which include physical contact between opponents (i.e., jump attack, attack/lunge, and tussle) occur at a significant rate during interactions (Table 3), but stalk-eyed flies do not appear to directly injure one another during aggressive interactions (Wilkinson and Dodson 1997). In terms of game theory models, SAM is characterized by physical contact between opponents with no injuries occurring but costs accruing through energy loss, predation risks, and time lost foraging or mating (Enquist et al. 1990). The E-WOA assumes that no physical contact occurs during interactions (Kelly 2006; Mesterton-Gibbons et al. 1996), and CAM assumes that physical contact leads to injuries during aggressive interactions that increase the rate of cost accrual to the eventual loser with lower RHP (Payne 1998). Thus, the presence of physical contact without apparent injuries is most consistent with SAM, is inconsistent with E-WOA, and equivocal at best for CAM (Kelly 2006).

Previous work conducted on stalk-eyed flies examined how contest duration was related to size asymmetries and winner and loser size to determine the model of contest settlement (Brandt and Swallow 2009; Panhuis and Wilkinson 1999). Initially, Panhuis and Wilkinson (1999) concluded that mutual assessment was the likely contest settlement model. However, re-analysis by Brandt and Swallow (2009) of the dataset showed that self-determined persistence may play a role in contest settlement in stalk-eyed flies. By utilizing sequential analysis, we were able to test key predictions of contest settlement models that are based on patterns of escalation, physical contact, and behavioral matching (Arnott and Elwood 2009; Briffa and Elwood 2009; Enquist and Leimar 1983; Hack 1997; Payne and Pagel 1997; Payne 1998). Based on predictions of escalation patterns, behavioral mismatching, and physical contact during interactions, our results with stalk-eyed flies are most consistent with SAM of contest settlement (Enquist and Leimar 1983; Payne 1998). Our analysis showed that during aggressive interactions, male stalk-eyed flies

Table 5 List of behavioral transitions that contributed significantly to the high chi-squared value for differences between contest winners and losers with retreat and pursue behaviors ($\chi_{22}^2=837.35$, $p<0.0001$) and without pursue and retreat behaviors ($\chi_{10}^2=22.05$, $p<0.0148$)

Behavioral transition	Chi-squared value	Winner transition (%)	Loser transition (%)
Retreat → end contest	190	50	83
Pursue → end contest	74	66	35
Flex → retreat	57	1.7	38
Line up eye stalks → rear up[a]	4.30	27	21

[a] Transitions without pursue and retreat behaviors in analysis

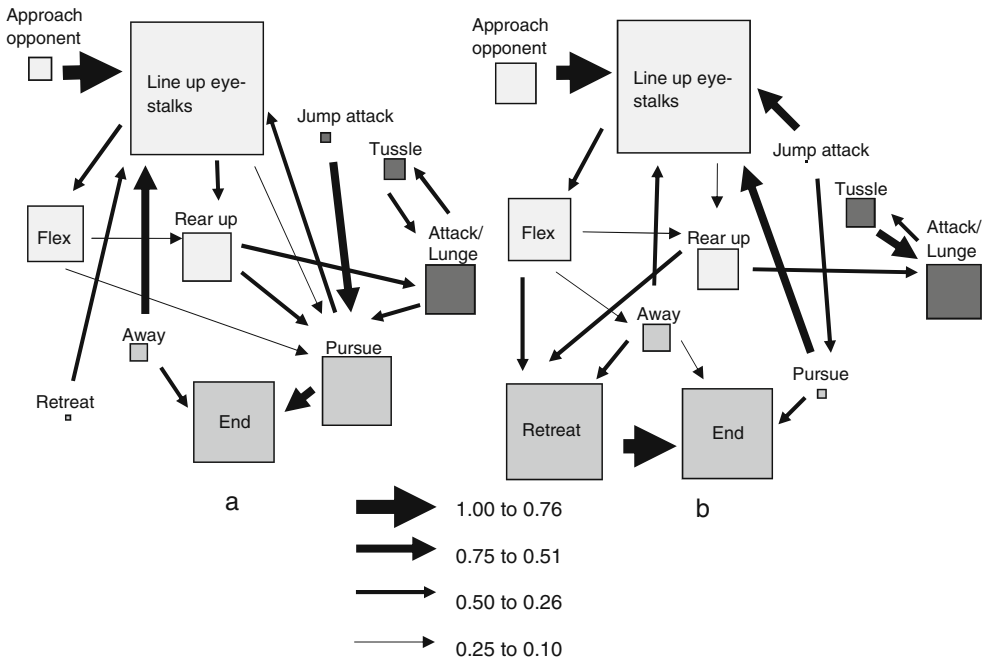


Fig. 3 Kinematic diagram of contest (**a**) winner and (**b**) loser behavioral transitions that occurred more frequently than expected by chance in the contests of male *T. dalmanni*. All transitions occurred greater than 10% of the time and are significant at $p<0.050$. *Box size* indicates frequency of behavior; *arrow thickness* indicates probability. *Light shading* is indicative of low-intensity behaviors, *dark shading* indicates high-intensity behaviors, and *medium shading* indicates conflict resolution behaviors

showed patterns of escalation, significantly transitioning from low-intensity behaviors to high-intensity behaviors without de-escalation. Both E-WOA and CAM are less consistent with this observation, as these two game theory models predict de-escalation within the interaction. When analyzing for patterns of behavioral matching between winners and losers, stalk-eyed flies did not show matching of behavioral transitions, even when controlling for scoring consequences of retreat and pursuit behaviors. This result is congruent with both SAM and CAM (Payne 1998; Enquist and Leimar 1983) models but is inconsistent with E-WOA which should have behavioral matching between opponents (Mesterton-Gibbons et al. 1996). Finally, our analysis conclusively shows that stalk-eyed flies escalate to behavior that involves physical contact but do not inflict detectable direct injuries onto their opponents. Physical contact corresponds with both SAM and CAM, but CAM predicts injuries to be inflicted onto the opponent, whereas SAM predicts no injuries. The E-WOA predicts no physical contact during interactions and thus is not concordant with our findings (Kelly 2006). Thus, our findings are most congruent with SAM, which is a type of mutual assessment during conflict resolution (Arnott and Elwood 2009; Briffa and Elwood 2009; Enquist and Leimar 1983; Payne 1998; Stuart-Fox 2006).

Conclusion

In this study, we examined the sequence of aggressive behaviors during male–male competition in stalk-eyed flies in order to distinguish between the mutual assessment hypothesis and the self-determined persistence hypothesis. After quantifying patterns of escalation, behavioral matching, and physical contact between opponents, we found that stalk-eyed fly conflict resolution is most consistent with the sequential assessment model (Enquist and Leimar 1983). The sequential assessment model makes sense with regard to dimorphic species of stalk-eyed flies. When males engage each other, they invariably line up with eye stalks nearly touching, which certainly appear to function as a mechanism to accurately assess or size up an opponent. The "size up" hypothesis was first proposed by McAlpine (1979) with regards to a different family of fly that also bears eye stalks (Platystomatidae). Furthermore, in all dimorphic species studied to date, female stalk-eyed flies accurately distinguish between and actively select males with longer eye stalks, indicating the ability to assess male size (Wilkinson 1993; Wilkinson and Dodson 1997). Since eye-stalk length is highly correlated with body size, a common proxy for resource holding potential, the most parsimonious explanation of data is that male stalk-eyed flies will also utilize eye stalks to reliably assess the strength of their opponents (Burkhardt and de la Motte 1983). Thus, information gathered from an opponent should be the evolutionary stable strategy, as assessment of the opponent's strength relative to one's own would reduce potential costs associated with increasingly escalated interactions. Previous work in stalk-eyed flies showing that loser RHP predicts contest duration suggests some degree of self-determined persistence, but the lack of a significant relationship between winner RHP and contest duration made distinguishing between mutual assessment and self-determined persistence difficult, since a correlation between winner RHP and contest

duration is consistent with both models (Arnott and Elwood 2009; Brandt and Swallow 2009). Therefore, further studies that consider both contest structure and contest duration components are needed to fully understand conflict resolution in stalk-eyed flies.

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Ethical standards The work in this study was carried out with the highest ethical standards according to the laws of the country in which the work was performed.

Conflicts of interest The authors declare they have no conflict of interest.

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