



Pure Self-Assessment of Size During Male–Male Contests in the Parasitoid Wasp *Nasonia vitripennis*

Yi-Jiun J. Tsai*, Edward M. Barrows*† & Martha R. Weiss*

* Department of Biology, Georgetown University, Washington, DC, USA

† Center for the Environment, Georgetown University, Washington, DC, USA

Correspondence

Yi-Jiun Jean Tsai, Department of Biology,
Georgetown University, Reiss 406, 37th and
O Streets, NW, Washington, DC 20057, USA.
E-mail: yjt3@georgetown.edu

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Abstract

During contest competition, a competitor may persist in a given contest based on information regarding its own fighting ability (resource-holding potential, RHP), or that of its opponent. Although a number of models formalize the ways in which competitors are hypothesized to use RHP-related information to determine their persistence in contests, we focused on pure self-assessment and mutual assessment models in this study. According to pure self-assessment models, a competitor uses only information regarding its own RHP to determine its persistence in a contest. In contrast, according to mutual assessment models, persistence is based on information regarding a competitor's RHP relative to that of its opponent and therefore requires assessment between competitors. In this study, using size as a proxy for RHP, we tested whether the parasitoid wasp *Nasonia vitripennis* utilizes pure self-assessment or mutual assessment during pairwise, male–male contests. When we examined competitors of varied sizes, we found that the losing male's size was positively related to contest duration, but the winning male's size was uncorrelated with contest duration. When we examined contests in which competitors were size-matched, we found that the mean size of paired competitors was positively related to contest duration. These results suggest that male *N. vitripennis* engage in pure self-assessment during contests.

Introduction

Contest competition is thought to select for traits that either increase the likelihood of fighting success or allow for alternative mating phenotypes (Andersson 1994; Andersson & Iwasa 1996). Generally, contest winners are thought to be those that possess greater fighting ability (resource-holding potential, RHP), value the contested resource more highly (resource value, RV), or both (Parker 1974; Enquist & Leimar 1983, 1987; Riechert 1998; Vieira & Peixoto 2013). Before contests, competitors may use information about themselves or that of their opponents to determine whether to participate. During contests, such information can be used to determine how long to persist. In many cases, this information pertains to a competitor's RHP, which is often related to traits such as body size, weaponry, or strength (Parker 1974).

Many models formalize the various ways in which competitors are hypothesized to assess RHP and predict how assessment determines persistence during contests. In this study, we focused on two main types of models that represent extremes on a continuum of assessment strategies: pure self-assessment and mutual assessment (Prenter et al. 2006; Arnott & Elwood 2009). In these cases, assuming that RV is constant between competitors, individuals that are able to persist for longer and choose to do so become the eventual contest winners (Taylor & Elwood 2003; Arnott & Elwood 2009; Briffa & Elwood 2009).

In pure self-assessment models, a competitor uses only information regarding its own RHP in determining its persistence in contests. The duration of a contest is thought to be determined by the time it takes an individual with lower RHP to reach its limit in fighting costs. Specifically, it is hypothesized that

competitors suffer costs as a result of their own participation in fighting (but not as a result of their opponents' actions), and those with lesser RHPs give up more quickly than those with greater RHPs because they reach their limits in time and energy first (Mesterton-Gibbons et al. 1996; Payne & Pagel 1996, 1997; Taylor & Elwood 2003; Arnott & Elwood 2009; Briffa & Elwood 2009). Pure self-assessment models include the 'war of attrition without assessment' model (Mesterton-Gibbons et al. 1996) and the 'energetic war of attrition' model (Payne & Pagel 1996, 1997). Species thought to utilize pure self-assessment during contests include orb-weaving spiders (*Metellina mengei*, Bridge et al. 2000), jumping spiders (*Plexippus paykulli*, Taylor et al. 2001), and amphipods (*Gammarus pulex*, Prenter et al. 2006).

In contrast, in mutual assessment models, a competitor gathers information about its opponent's RHP and compares it to information regarding its own RHP. In this case, a competitor can more easily assess an opponent's RHP relative to its own when the difference in RHP between competitors is large. Therefore, larger RHP differences between competitors can lead to shorter contests, as the weaker competitor is able to detect its own 'inferiority' more quickly, withdraw from the contest, and minimize its costs of fighting. However, when the differences between competitor RHPs are small, it can be more difficult to determine which competitors have greater RHPs, so more sampling is needed to detect these differences and therefore contests last longer (Maynard Smith & Parker 1976; Parker & Rubenstein 1981; Enquist & Leimar 1983; Enquist et al. 1990; Taylor & Elwood 2003; Arnott & Elwood 2009). Mutual assessment models include the 'sequential assessment' model (Enquist & Leimar 1983; Enquist et al. 1990) and some 'asymmetric war of attrition' models (Maynard Smith & Parker 1976; Parker & Rubenstein 1981; Hammerstein & Parker 1982). Species thought to utilize mutual assessment during contests include cichlids (*Archocentrus nigrofasciatus*, Leiser et al. 2004), landmark-defending wasps (*Hemipepsis ustulata*, Kemp et al. 2006), and hermit crabs (*Pagurus middendorffii*, Yasuda et al. 2012).

In this study, we asked whether males use pure self-assessment or mutual assessment of RHP during contests in the parasitoid wasp *Nasonia vitripennis*, using body size as a proxy for RHP. To distinguish between these two types of models, we compared our data to model-specific predictions outlined in seminal papers by Taylor & Elwood (2003) and Arnott & Elwood (2009). These predictions pertain to two types of contests, contests between randomly paired

competitors and contests between size-matched competitors.

In contests between randomly paired competitors, pure self-assessment can be distinguished from mutual assessment by examining the relationship between contest duration and the absolute sizes of both the losing and winning competitor (Taylor & Elwood 2003; Arnott & Elwood 2009). Under pure self-assessment, the loser's size is expected to strongly and positively correlate with contest duration (Fig. 1a), while the winner's size should either weakly and positively correlate or be uncorrelated with contest duration (Fig. 1d; Mesterton-Gibbons et al. 1996; Taylor & Elwood 2003). These two relationships clearly reflect the underlying idea of pure self-assessment, in which persistence is driven solely by the losing individual's limitations in time and energy, and the winning competitor's limits are never reached (Mesterton-Gibbons et al. 1996; Payne & Pagel 1997; Taylor & Elwood 2003). Under mutual assessment, the loser's size should strongly and positively correlate with contest duration (Fig. 1b), as it does under pure self-assessment. In contrast, the winner's size should strongly and negatively correlate with contest duration (Fig. 1e). In this case, the differences in size between competitors (a function of both competitors' absolute sizes) drives contest persistence, with smaller differences taking longer to assess and leading to longer contests than larger differences (Parker & Rubenstein 1981; Enquist & Leimar 1983; Enquist et al. 1990). As eventual losers increase in size, they engage in longer contests because size differences between them and their competitors decrease. On the other hand, as eventual winners increase in size, they engage in shorter contests because size differences between them and their competitors increase (Briffa & Elwood 2009). Overall, the distinguishing feature between pure self- and mutual assessment in contests between randomly paired competitors is the relationship between the winner's size and contest duration (Arnott & Elwood 2009).

In contests between size-matched competitors, the mean size of paired competitors is expected to correlate positively with contest duration under pure self-assessment (Arnott & Elwood 2009; Fig. 1g). Contests between small, paired competitors are shorter than those between large, paired competitors because the limits of small, losing competitors are reached before those of large, losing competitors (Mesterton-Gibbons et al. 1996; Payne & Pagel 1996, 1997). In contrast, under mutual assessment, the mean size of paired competitors is expected to be uncorrelated with contest duration (Arnott & Elwood 2009; Fig. 1h). In this

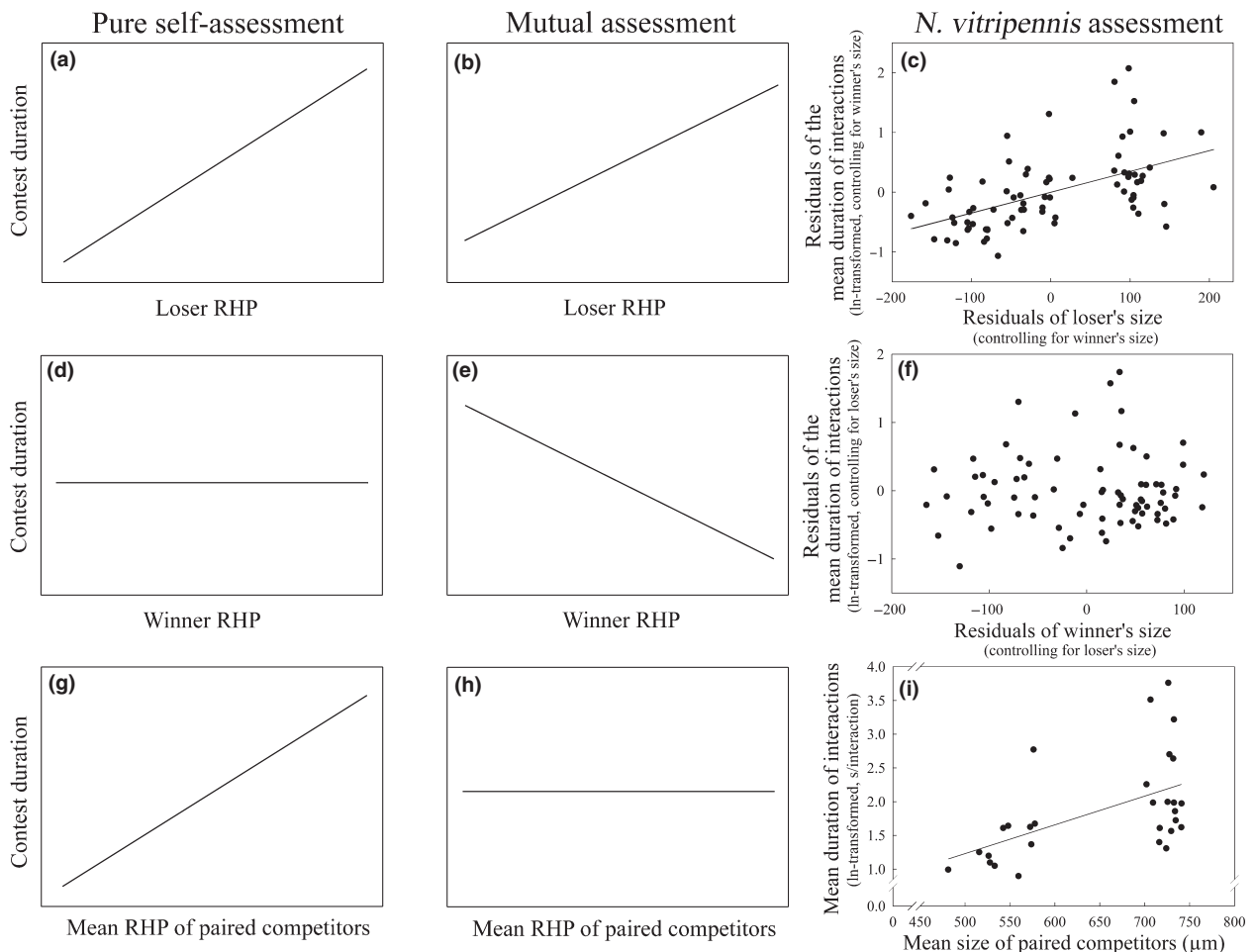


Fig. 1: Panels a, d, and g show predictions for pure self-assessment models. Panels b, e, and h show predictions for mutual assessment models. These predictions show the relationship between contest duration and either the loser's resource-holding potential (RHP) (a, b), the winner's RHP (d, e), or the mean RHP of paired, RHP-matched competitors (g, h). Note that panels a, b, d, and e reflect the relationships expected using multiple regression, in contrast to figures by Arnott and Elwood (2009) showing relationships expected using simple regression. Under pure self-assessment, winner RHP and contest duration are expected to be positively correlated with one another when using simple regression, but be uncorrelated when taking loser RHP into account using multiple regression (d; as recommended by Taylor & Elwood 2003). Relationships in a, b, and e (adapted from Arnott & Elwood 2009 and Taylor & Elwood 2003, reprinted with permission from Elsevier) are the same whether using simple or multiple regression. Panels c, f, and i show the relationships between contest duration and competitor size (hind tibia length) in *N. vitripennis* and match predictions for pure self-assessment. In contests between males of varied sizes, the ln-transformed mean duration of interactions was positively related to the loser's size (c, partial regression plot), but was unrelated to the winner's size (f, partial regression plot). In contests between size-matched competitors, the ln-transformed mean duration of interactions was positively related to the mean size of paired competitors (i). Although males varied in size across all trials, mid-sized males (580–700 μm in hind tibia length) were excluded from the analysis of size-matched competitors because we paired males based on size categories (determined by foundress-to-host ratios), in which only large–large and small–small males were similar in size.

case, the difference in size between competitors is similar between pairs, so each pair will require a similar amount of time for assessment, regardless of the competitors' absolute sizes (Maynard Smith & Parker 1976; Parker & Rubenstein 1981; Enquist & Leimar 1983; Enquist et al. 1990). Thus, pure self-assessment can be distinguished from mutual assessment in contests between size-matched competitors by examining the relationship between contest duration and the

mean size of paired competitors (Arnott & Elwood 2009).

Given these predictions, we tested whether male *N. vitripennis* use pure self-assessment or mutual assessment during contest competition by observing pairwise contests among males of varied sizes and determining the relationship between contest duration and the winning male's size. We also examined a subset of these data, in which competitors were

size-matched, and determined the relationship between contest duration and the mean size of paired competitors. In each case, we determined whether the observed relationships between contest duration and competitor sizes matched the predictions corresponding to pure self-assessment or mutual assessment.

Methods

Study Species

Distributed worldwide, *N. vitripennis* (Chalcidoidea: Pteromalidae) is a solitary (non-eusocial) parasitoid wasp that lays 15–30 eggs per host (Whiting 1967). *N. vitripennis* feeds on the pupae of a wide range of dipteran species and is often found with its dipteran host in bird nests and carrion (Werren 1983; Abraham 1985). As adults, males usually emerge from host puparia before females and interact agonistically for access to host puparia from which females will eventually emerge (King et al. 1969; van den Assem et al. 1980; Moynihan & Shuker 2011). In general, agonistic interactions between males begin with orientation toward competitors, antennation, mandible flaring, and wing raising. These interactions can then escalate into physical behaviors, in which one male may grab or charge the other. The recipient of this escalation may respond with further agonism, fleeing from the aggressor, or staying still (van den Assem et al. 1980; Leonard & Boake 2006). In addition to direct competition, males may also deposit substrate-borne pheromones that attract females, although this tactic is thought to be secondary to the dominance structure established by contests at the host puparia (Ruther et al. 2007; Steiner & Ruther 2009; Blaul & Ruther 2012).

In *N. vitripennis*, body size predicts outcomes in pairwise contests, in which larger males are more likely to win contests than smaller males (Tsai et al. 2014). While there is a clear effect of body size on contests outcomes, papers by Burton-Chellew et al. (2007), Moynihan & Shuker (2011), and Blaul & Ruther (2012) suggest that larger males do not experience greater mating or reproductive success when competing against smaller males for access to adult females. However, while informative, these experiments are unlikely to reflect the mating and reproductive success of such males in nature where males do not guard freely moving females, but instead compete for access to monopolizable, female-containing host puparia (King et al. 1969; van den Assem et al. 1980). Thus, body size plays a clear role in

determining contest success among male *N. vitripennis*, but whether these effects translate into mating and reproductive success is unclear.

Rearing Conditions

We obtained wildtype *N. vitripennis* from Ward's Natural Science (Rochester, New York, NY, USA). We reared all wasps under 16:8 hour light:dark conditions under fluorescent lights and maintained them at $23 \pm 0.7^\circ\text{C}$ minimum and $27 \pm 0.8^\circ\text{C}$ maximum temperatures and $50 \pm 10\%$ humidity (mean \pm SD). Temperature and humidity fluctuated throughout the day, mirroring natural, outdoor conditions. All individuals were reared in translucent 29.6-ml (1-oz) plastic cups with clear plastic tops (Solo; Dart Container Corporation, Lake Forest, IL, USA).

Female *N. vitripennis* pupae obtained directly from Ward's were reared into adulthood and then given *Sarcophaga bullata* host pupae in which to oviposit and produce experimental individuals. We generated male offspring of varied sizes by manipulating the foundress-to-host ratio. Increased foundress-to-host ratios result in increased larval competition and yield smaller offspring (Nagel & Pimentel 1963; Wylie 1965; Whiting 1967). Thus, we gave either four hosts to one foundress for 3 d to produce large male offspring, or one host to four foundresses for 3 d to produce small male offspring (Burton-Chellew et al. 2007). Foundresses received host(s) and honey solution on the day of their eclosion. Host(s) was replaced on days 3 and 6 post-eclosion, and females oviposited throughout this period. Parasitized hosts were incubated for 11 d and then refrigerated (-4°C) until the start of the experiment, at which time *N. vitripennis* pupae were removed from their host puparia and reared individually to adulthood. Refrigeration slowed *N. vitripennis* development and allowed for control over the timing of eclosions. Further details regarding male offspring sizes are reported in Tsai et al. (2014).

Contest Competition Trials

We examined male–male contest competition in *N. vitripennis* by observing 2-d-old males in paired contests ($n = 80$). One day prior to testing (1 d post-eclosion), we marked males for individual identification using acrylic paint (red or white, randomly assigned). After marking, we randomly assigned non-sibling males to competing pairs of large–large (one foundress to four hosts), large–small, or small–small (four foundresses to one host) sizes. Although we paired males based on the foundress-to-host ratios

from which they were derived, large and small males varied in size within their categories. We chose to use absolute size (hind tibia length) as a continuous variable in statistical analyses (described below) to more precisely account for the variation in competitor size.

Males were completely isolated as adults and therefore had no previous contest experience prior to a trial. For the trial, we placed paired males into a 60 × 15 mm polystyrene Petri dish (Falcon, Becton Dickinson and Company, Lincoln Park, NJ, USA) that was 22 cm from a 75-watt incandescent light. Trials lasted 10 min, starting immediately after we introduced paired males into their trial arenas. Paired competitors fought in 76 of 80 trials conducted, even in the absence of any host- or female-related cues. All trials were video-recorded using a full HD camcorder (Xacti Sanyo, Panasonic, San Diego, CA, USA) positioned directly above the arena.

Measures of Winning, Duration of Interactions, and Body Size

After all trials were completed, we scored videos for contest-related interactions (partial ethogram in Tsai et al. 2014) while blind to treatment. We examined only interactions that included physical behaviors and defined an interaction as beginning with a physical behavior (grab or charge) and ending when neither competitor was facing or in physical contact with the other. The loser of an interaction was the individual that remained still or fled at the end of the interaction. The winner of an interaction was by default the other competitor. In cases where an interaction ended with the loser staying still, the winner was the last competitor to grab or charge his opponent (Video S1). In cases where an interaction ended with the loser fleeing from its competitor, the winner was the individual that chased its competitor or was last to face in the direction of his opponent (Video S2). Using these criteria, a winner and loser could be assigned unambiguously to 99% of all completed interactions. An interaction could comprise multiple behaviors, and a trial could include multiple bouts of interaction between two competitors (Tsai et al. 2014).

In some interactions, males engaged in an abdomen touching behavior, in which a male grabbing his opponent would touch his abdomen to that of his opponent. In many cases of abdomen touching, both males remained still for long periods of time, and the behavior did not appear to be agonistic. Of 913 total interactions scored, abdomen touching occurred in 33 interactions (3.6%). The duration of abdomen touching within a given interaction ranged from 1 to

124 s (mean ± SD: 21.4 ± 33.7 s). We therefore excluded the time in which males engaged in abdomen touching to avoid artificially inflating the duration of agonistic interactions.

From these scorings, we (1) calculated the duration of the very first interaction that occurred between paired competitors (s), (2) calculated the mean duration of all interactions that occurred during a 10-min trial (s per interaction), and (3) determined the winner of a given trial. We calculated the mean duration of interactions by dividing the total duration of all completed interactions by the total number of completed interactions that occurred during a trial. The trial winner was defined as the individual that won the majority of all interactions within a trial. If each competitor won an equal number of interactions, the trial was considered to have ended in a tie and was therefore omitted from the analysis of winner and loser sizes, but not from the analysis of size-matched competitors.

To measure body size, we used hind tibia length, which is highly correlated with mass (Tsai et al. 2014). After each trial, experimental individuals were frozen at -15 to -20°C until they could be dissected. Both hind legs of each male were mounted in glycerol on a slide. The mounts were then photographed using a Zeiss Axiophot microscope (Carl Zeiss Inc., Oberkochen, Germany) at 10× magnification and using Openlab software (PerkinElmer, Waltham, MA, USA). We then measured hind tibia lengths from the photographs using ImageJ software (National Institutes of Health, Bethesda, MD, USA). To ensure accuracy, both hind tibiae were measured for each individual, and the mean tibia length for an individual was used in all statistical analyses (Tsai et al. 2014).

Statistical Analyses

Outcome of contests between males of varied sizes

We tested whether a competitor's probability of winning a trial was affected by his size or marked color using a generalized linear mixed-effects logistic regression (binomial-error structure and logit-link function), in which a competitor's winning or losing a trial was the response variable, the color he was marked (red or white) was the predictor variable, his tibia length was a covariate, and the pair or trial in which he competed was the random factor.

Duration of contests between males of varied sizes

To examine whether the winner's size, loser's size, or both explained contest persistence, we used multiple regressions. The explanatory variables were the trial

winner and the trial loser's hind tibia lengths, and the response variable was either the duration of the first interaction or the mean duration of interactions that occurred during a trial (ln-transformed to meet assumptions of homoscedasticity).

Duration of contests between size-matched males

To examine contests between size-matched competitors, we used a subset of the data, in which competitors differed in tibia length by $<30 \mu\text{m}$. The difference between an individual's right and left tibia never exceeded $30 \mu\text{m}$ ($n = 135$, mean difference between tibiae within an individual \pm SD: $6 \pm 6 \mu\text{m}$, range: $0\text{--}25 \mu\text{m}$). Thus, the difference in hind tibia length between size-matched competitors was similar to the measuring error within an individual. We used simple regressions, in which the explanatory variable was the mean size of paired competitors and the response variable was either the duration of the first interaction or the mean duration of interactions (ln-transformed to meet assumptions of homoscedasticity).

All statistical analyses were conducted using R (R Core Team 2013) and RStudio (2013).

Results

Of 80 trials, competitors interacted agonistically for more than 1 s in 75 trials and did so at a rate of 12.2 ± 9.0 completed interactions per trial (mean \pm SD; range = 1–56 interactions). During a 10-min trial, competitors interacted agonistically for a total of 54.6 ± 36.1 s (mean \pm SD; range = 5–213 s, $n = 75$ trials). The duration of the first interaction was 8.8 ± 8.7 s (mean \pm SD; range = 1–51 s, $n = 72$ trials). The mean duration of all interactions per trial was 6.3 ± 6.6 s/interaction (mean \pm SD; range = 1.6–43 s/interaction, $n = 75$ trials).

Outcome of Contests between Males of Varied Sizes

A competitor's probability of winning was not affected by his marked color (GLMM: $n = 148$ competitors in 74 trials, $\beta = -0.218$, $\text{SE} = 0.348$, Wald $\chi^2 = 0.391$, $p = 0.532$), but was affected by his size ($\beta = 0.007$, $\text{SE} = 0.002$, Wald $\chi^2 = 14.918$, $p = 0.0001$), in which larger males had a higher probability of winning than smaller males (see Tsai et al. 2014).

Duration of Contests between Males of Varied Sizes

The duration of the first interaction between two competitors was explained by the loser's size

($\beta = 0.031$, $\text{SE} = 0.011$, $t = 2.929$, $p = 0.005$), but not by the winner's size ($\beta = 0.002$, $\text{SE} = 0.013$, $t = 0.137$, $p = 0.892$) in our model (multiple regression: $n = 71$, $R_{\text{adj}}^2 = 0.107$, $F_{2,68} = 5.19$, $p = 0.008$). Similarly, the ln-transformed mean duration of all interactions occurring during a 10-min trial was explained by the loser's size (Fig. 1c, $\beta = 0.004$, $\text{SE} = 0.0007$, $t = 5.296$, $p < 0.0001$) but not by the winner's size (Fig. 1f; $\beta = 0.0002$, $\text{SE} = 0.0008$, $t = 0.237$, $p = 0.813$) in our model (multiple regression: $n = 74$, $R_{\text{adj}}^2 = 0.292$, $F_{2,71} = 16.02$, $p < 0.0001$). One trial resulted in a tie between the competitors and was excluded from these analyses. The hind tibia length of trial winners was $663 \pm 80 \mu\text{m}$ (mean \pm SD; range = 472–766 μm , $n = 74$), while that of trial losers was $601 \pm 101 \mu\text{m}$ (range = 432–762 μm , $n = 74$). The difference in hind tibia length between paired competitors ranged from 1 to 290 μm .

Duration of Contests between Size-Matched Males

Among size-matched competitors, the mean size of paired competitors did not predict the duration of the first interaction (regression: $n = 29$, $\beta = 0.039$, $\text{SE} = 0.023$, $R_{\text{adj}}^2 = 0.063$, $F_{1,27} = 2.879$, $p = 0.101$). However, the mean size of paired competitors was positively related to the ln-transformed mean duration of all interactions (Fig. 1i; regression: $n = 29$, $\beta = 0.004$, $\text{SE} = 0.001$, $R_{\text{adj}}^2 = 0.257$, $F_{1,27} = 10.69$, $p = 0.003$). The hind tibia length of paired, size-matched competitors was $651 \pm 93 \mu\text{m}$ (mean \pm SD; range = 481–741 μm , $n = 29$).

Discussion

We examined whether *N. vitripennis* use pure self- or mutual assessment strategies during male–male contests by testing predictions regarding the relationship between competitor size and contest duration. We found that the losing male's size was positively related to both the duration of the first interaction and the mean duration of all interactions, but that the winning male's size was uncorrelated with either measure of duration. Therefore, a contest lasted for as long as the losing male persisted, regardless of the winning male's size. When competitors were size-matched, the mean size of paired competitors was unrelated to the duration of the first interaction, but was positively related to the mean duration of all interactions. Thus, competitors persisted based on absolute rather than relative size, although this was detected only when using the mean across multiple interactions. Together, these results are consistent with predictions of pure

self-assessment, and they indicate that individuals take into account only information regarding their own sizes, and not those of their opponents, during contests (Taylor & Elwood 2003; Arnott & Elwood 2009).

While *N. vitripennis* exhibits pure self-assessment during contests, other hymenopteran species vary in the type of assessment used before or during contests. For example, some hymenopterans exhibit mutual assessment. Male fig wasps (*Idarnes* spp.) are thought to use display behavior to measure their opponent's mandible gape width and thereby determine their participation in a given fight (Pereira & Prado 2005), although these conclusions were based on qualitative, observational data. Similarly, male landmark-defending wasps (*H. ustulata*; Kemp et al. 2006) persist in non-contact aerial displays based on the relative size difference between paired competitors. In contrast, other species appear to use pure self-assessment. Honeybee (*Apis mellifera*) queens are less likely to fight other queens within the same hive when their fighting ability has been experimentally diminished (ablated mandibles), suggesting the occurrence of self-, but not mutual, assessment (Dietemann et al. 2008). Among *Sycoscapter* (species A) fig wasps, males do not appear to assess their competitors before or during fights; the difference in head width and mandible length between fighting males does not differ from that of randomly chosen males, and contest duration is correlated with the head width and mandible length of the loser, but not the winner (Moore et al. 2008). In some instances, the occurrence of assessment may even differ between different populations of the same species. Whereas North American populations of paper wasps (*Polistes dominulus*) use mutual assessment when choosing between rivals based on the visual cue of clypeal facial spots (Tibbetts & Dale 2004; Tibbetts et al. 2010), European populations do not (Green & Field 2011).

Given that mutual assessment could help competitors avoid contests or minimize the costs of contests, and given its occurrence in other hymenopteran species, why does *N. vitripennis* not utilize mutual assessment during physical contests? There are a number of possible explanations for the occurrence of self-assessment, rather than mutual assessment, including that (1) *N. vitripennis* males lack the sensory abilities needed for assessment (Taylor & Elwood 2003); (2) males lack reliable signals that could be used for such assessment (Taylor & Elwood 2003; Elias et al. 2008); and (3) assessment of competitors is too costly in terms of time and energy (Elias 2008). It seems unlikely that male *N. vitripennis* lack the sensory abilities

needed for mutual assessment, given that they can respond to a variety of odor and color cues. For example, males respond to female cuticular hydrocarbons (Steiner et al. 2006) and to pheromones released by other males (Ruther et al. 2011), as well as learn to associate color with mates (Baeder & King 2004). With regard to RHP-related signals, it is unclear whether competitors possess reliable signals that could be used for assessment. Male *N. vitripennis* engage in display behaviors such as antennation, mandible flaring, and wing raising (van den Assem et al. 1980) that could allow for the signaling of size prior to a contest. However, males engaged in physical interactions at least once during a 10-min period in 95% of all trials conducted, suggesting that males eventually escalated their interactions, regardless of display behavior. Studies explicitly examining display behavior in *N. vitripennis* are needed to elucidate its role in contests. Regarding the costs of assessment, it is possible that the time and energy needed for mutual assessment may be too costly to occur in *N. vitripennis*. In nature, males may encounter a wide range of competitor densities, depending on host patch size and brood sex ratio, which can range from being heavily female-biased to all-male (derived from unmated foundresses; Whiting 1967; Grillenberger et al. 2008). At high male densities, contest competition appears to break down into scramble competition (van den Assem et al. 1980) such that males can no longer successfully defend their territories. Thus, at high male densities, the high frequency with which competitors are encountered may make a strategy such as mutual assessment costly in time and energy, especially as there may be little payoff for winning contests under these circumstances.

Our study was conducted under artificial conditions, in which no female-containing hosts or emerging females were present. These stimuli are clearly not necessary to evoke contests, and their absence allows for the quantification of measures such as contest duration (e.g., without interruptions due to mating attempts). However, future studies that include naturally occurring, female-containing host puparia could provide more insight into the role of body size in contests, and we are currently conducting these tests. Furthermore, studies examining potential changes in assessment strategy during displays, during different stages of escalation, and across successive interactions either within a given trial or across different opponents could also provide intriguing insight into the potential for more complex assessment strategies (Whitehouse 1997; Elias et al. 2008; Hsu et al. 2008; Yasuda et al. 2012). In addition to RHP, RV also

plays an important role in determining contest outcomes (Parker 1974; Maynard Smith & Parker 1976). Thus, studies examining whether *N. vitripennis* assesses RV would be of interest, especially considering that RV assessment occurs in other parasitoid wasps (Humphries et al. 2006; Stockermans & Hardy 2013).

We provide evidence for the occurrence of pure self-assessment of RHP in *N. vitripennis*. Although the role of RHP in contests is already well studied in many parasitoid species (Hardy et al. 2013), only a few studies, including ours, examine assessment of RHP by competitors during contests. In doing so, this study furthers our understanding of contest competition.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Video S1: Video of an interaction ending with the loser staying still.

Video S2: Video of an interaction ending with the loser fleeing.