GABRIEL LOBREGAT DE OLIVEIRA

FIGHTING ASSESSMENT STRATEGIES: MODEL EVALUATION AND EMPIRICAL EVIDENCES FROM A NEOTROPICAL CRICKET

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ecologia, para obtenção do título de *Doctor Scientiae*.

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(Orientador)

Para Benjamin, minha fonte de alegria e de esperança em um futuro melhor.

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SUMÁRIO

ABSTRACT

OLIVEIRA, Gabriel Lobregat, D.Sc., Universidade Federal de Viçosa, July, 2018. **Fighting assessment strategies: model evaluation and empirical evidences from a Neotropical cricket.** Adviser: Carlos Frankl Sperber. Co-advisers: Paulo Enrique Cardoso Peixoto and Thiago Gechel Kloss.

Animal contests usually end before the death of one of the rivals, and their resolution depends on the decision of the loser to withdraw. Evolutionary game theory models assume two major asymmetries between contestants as determinants for fighting success: fighting ability and motivation to win the contest. Different models propose strategies of how contestants assess information about these asymmetries in their decision to stay or leave the fight. The empirical evaluation of the relationship between proxies of fight-associated costs and fighting ability allows to discriminate which of these models explain better the contest behavior of species. Empirical studies also try to elucidate the mechanisms that contestants use to transmit information to the opponent by evaluating the information content of aggressive displays and other agonistic behaviors. In the first chapter of this thesis, we used individuals from simulated populations to establish contests that followed the predictions of different assessment strategies. We demonstrated that different offensive capacities (i.e. damage output) in injurious contests might generate similar results for different assessment models, hampering the discrimination of which model explain better the contests. We also showed that when contest behavioral phases follow different assessment strategies, the evaluation of overall contest alone hinders the detection of what assessment strategy is applied in the second phase, as every contest present the first phase, but not all contests escalate to the second phase. Thus, it is necessary to consider the offensive capacity of a species and the possibility of switching assessment between contest phases to correctly assign what assessment strategies explain the contest behavior of species. In the second chapter, we investigated the contest behavior of *Melanotes ornata* in order to elucidate what assessment strategy these crickets use. We showed that, as expected by the results of Chapter 1, a single assessment strategy does not explain the contest dynamics of this species. We found evidences that in the first contest phase, individual mutually assess each other and escalate to the second phase when their fighting abilities are similar. In the second contest phase, however, no fighting ability assessment appears to occur. We suggest that after escalation other asymmetries, like differences in motivation, may be more important to contest resolution. In the third chapter, we evaluated the information content of aggressive sound signals emitted by individuals in escalated phases. We show that sound parameters of aggressive song do not have any information about fighting ability. In counterpart, contestants with higher motivation showed a higher signaling effort along the contest and also displayed with a higher pulse rate. Thus, such signals contain information about motivational status of contestants and may be used in the assessment of opponent's motivation in escalated fights.

RESUMO

OLIVEIRA, Gabriel Lobregat, D.Sc., Universidade Federal de Viçosa, julho de 2018. **Estratégias de avaliação de luta: avaliação de modelos e evidências empíricas de um grilo Neotropical.** Orientador: Carlos Frankl Sperber. Coorientadores: Paulo Enrique Cardoso Peixoto e Thiago Gechel Kloss.

Conflitos animais geralmente acabam antes da morte de um dos rivais e, portanto, sua resolução depende da decisão do perdedor em sair. A Teoria de Jogos Evolutiva assume duas principais assimetrias entre combatentes como determinantes do sucesso na luta: a habilidade de luta e a motivação em vencer o conflito. Diferentes modelos propõem estratégias de avaliação alternativas em que combatentes utilizam informações sobre essas assimetrias na sua decisão de permanecer ou sair da luta. Estes modelos diferem na relação entre *proxies* de custos associados à luta e *proxies* da habilidade de luta e, portanto, permitem a avaliação de qual modelo se encaixa ao comportamento agonístico das espécies. Estudos empíricos também tentam elucidar os possíveis mecanismos que rivais utilizam para transmitir e receber informações entre eles, ao avaliar o conteúdo contido nos *displays* de agressividade e outros comportamentos agonísticos. No primeiro capítulo desta tese, nós utilizamos indivíduos de populações simuladas para estabelecer conflitos seguindo predições de diferentes estratégias de avaliação. Nós demonstramos que, ao alterar a capacidade ofensiva (ou seja, a relação entre o tamanho dos indivíduos e o dano que eles causam) em conflitos com imposição de injúrias, lutas seguindo o *Cumulative assessment model* podem gerar predições empíricas similares a de outros modelos de avaliação. Também demonstramos que, quando diferentes fases comportamentais dos conflitos seguem diferentes estratégias de avaliação, investigar uma única estratégia de avaliação para a luta inteira pode mascarar a detecção de qual estratégia é realizada na segunda fase da luta. Desta forma, é importante considerar a capacidade ofensiva de uma espécie e a possibilidade de suas lutas apresentarem trocas de estratégias, de forma a permitir uma correta determinação do mecanismo de avaliação utilizado em comportamentos de luta. No segundo capítulo, nós investigamos o comportamento agonístico de *Melanotes ornata* para determinar qual estratégia de avaliação estes grilos utilizam. Demonstramos que, como esperado pelos resultados do Capítulo 1, uma única estratégia de avaliação não consegue explicar a dinâmica de luta desta espécie. Nós encontramos evidências de que na primeira fase da luta, rivais se avaliam mutuamente e escalonam a luta para a segunda fase quando suas habilidades de luta são similares. No entanto, na segunda fase nenhuma estratégia de avaliação da habilidade de luta parece ocorrer. Desta forma, sugerimos que, quando as lutas escalonam, outras assimetrias como diferenças na motivação podem ser mais importantes na resolução dos conflitos. No terceiro capítulo, avaliamos quais informações os sinais acústicos agressivos emitidos nas lutas escalonadas podem conter. Nós demonstramos que os parâmetros sonoros do som de agressividade não contêm informações sobre a habilidade de luta. Em contrapartida, indivíduos com maior motivação em lutar apresentaram um maior esforço de sinalização ao longo do conflito e também emitiram som com uma maior taxa de pulsos. Portanto, concluímos que estes sinais contêm informações sobre o estado motivacional dos indivíduos e podem ser usados na avaliação da motivação do oponente em lutas escalonadas.

GENERAL INTRODUCTION

Sexual selection has been considered the main path to explain the evolution of the complex behaviors associated to agonistic interactions between males and mate choice by females (Lailvaux & Irschick 2006; Irschick et al. 2007). According to it, certain males present a mating advantage over others through the victory of agonistic interactions for sexual resources or due to their higher attractiveness to females (Berglund et al. 1996; Andersson and Simmons 2006; Cornwallis and Uller 2010; Lailvaux & Irschick 2006; Irschick et al. 2007). Such advantage is conferred by morphological and behavioral traits that ensure superiority in fights between males and/or are preferentially selected by females in their mating decisions (Berglund et al. 1996; Irschick et al. 2007; Dennenmoser and Christy 2013).

Particularly in competition for mates, theoretical and empirical studies aim to determine what attributes confer a fight advantage to competitors (Vieira and Peixoto 2013; McCullough 2014; Yasuda and Koga 2016), and how such competitors may use information about these attributes in their decision to stay in combat or withdraw from the fight (Arnott and Elwood 2008; Arnott and Elwood 2009). Evolutionary game theory is a very useful tool to evaluate these questions, as it deals with conflict of interest situations, like animal contests, where the success of an individual will greatly depend on the actions of its rival (Parker 1974; Maynard-Smith 1982). According to it, individuals primarily base their decisions to continue in the fight or withdraw from it on the relative costs and benefits of staying in combat (Maynard-Smith 1982). Thus, it is expected that animals alter their agonistic behavior according to the inherent value of the contested resource and to the potential injury, energetic and time costs of fighting (Enquist and Leimar 1983; Enquist and Leimar 1987; Payne and Pagel 1996; Payne 1998).

The first game theory models, like the Hawk-Dove game, were mainly interested to demonstrate how game theory reasoning could be applied to the logic of animal contests, but they considered unrealistic situations where contestants were totally symmetrical and could only employ one behavioral action in their contests (Maynard-Smith 1982). Latter models were developed with more realistic scenarios where rivals present asymmetries in their fighting ability and motivation to win, and where their fight strategy is not discrete, but a continuum of possible behavioral actions that are employed according to the information individuals have in a given moment (Hammerstein and Parker 1982; Enquist and Leimar 1983; Enquist and Leimar 1987; Mesterton-Gibbons et al. 1996; Payne and Pagel 1996; Payne 1998). The major difference between these models is what sort of information contestants assess in their decisions along the fight. According to them, three major assessment strategies may be employed in animal contests. Pure self-assessment states that individuals base their decision to stay or leave the fight solely on a self-threshold of costs associated to their fighting ability (Payne and Pagel 1996). Cumulative assessment states that injuries imposed by rivals increase the rate at which individuals reach their cost-thresholds, and as the fighting ability of an individual increases, so does their capacity to impose higher injury costs (Payne 1998). In counterpart, mutual assessment considers that individuals do not fight according to a cost-threshold. Instead, contestants gather information not only about themselves but also about their opponents in order to assess their relative inferiority (Enquist and Leimar 1983).

Since the development of such models, many empirical studies have evaluated which of them could better explain the contest behavior of animals (Arnott and Elwood 2008; Arnott and Elwood 2009). The main adopted approach for this has been the evaluation of the relationship between proxies of fight-associated costs and proxies of the individuals' ability to pay these costs (Taylor and Elwood 2003; Elwood and Arnott

2012). However, many studies found evidences to support more than a single model or no model at all (Arnott and Elwood 2009; Briffa and Elwood 2009), and some theoretical studies have argued that such models may be not able to depict the complexity that animal contest behavior may present (Elwood and Arnott 2012; Fawcett and Mowles 2013; Briffa and Lane 2017; Lane and Briffa 2017). Owing to such problems, recent studies have been suggesting new approaches to elucidate what assessment strategy better explain the contest behavior of species (Hsu et al. 2008; Judge and Bonanno 2008).

In this thesis, we evaluated the predictions of assessment strategy models with simulated data and observations of the contest behavior of a Neotropical cricket species. We also investigated what sort of information these crickets may provide with their aggressive acoustic displays emitted along escalated phases of their contests. In the first chapter, we used a simulation approach to demonstrate how different offensive capacities and different assessment strategies at behavioral phases may alter the empirical predictions of assessment strategies, hindering the correct assignment of assessment models to empirical data. In the second chapter, we incorporated the possibility of different assessment strategies in the contest phases of the cricket *Melanotes ornata* (Orthoptera: Phalangopsidae) and demonstrated that a single assessment strategy is not able to explain the entire contest of this species. Finally, in the third chapter, we analyzed what sort of information may be transmitted with the acoustic signals these crickets emit in their escalated phases. We showed that more motivated contestants present a higher signaling effort and emit signals with a higher pulse rate than non-motivated individuals, suggesting that the aggressive song of these species may be used by contestants to assess the motivational state of their rivals.

REFERENCES

Andersson M, Simmons LW. 2006. Sexual selection and mate choice. Trends Ecol Evol. 21:296–302.

Arnott G, Elwood RW. 2008. Information gathering and decision making about resource value in animal contests. Anim Behav. 76:529–542.

Arnott G, Elwood RW. 2009. Assessment of fighting ability in animal contests. Anim Behav. 77:991–1004.

Berglund A, Bisazza A, Pilastro A. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. Biol J Linn Soc. 58:385–399.

Briffa M, Elwood RW. 2009. Difficulties remain in distinguishing between mutual and self-assessment in animal contests. Anim Behav. 77:759–762.

Briffa M, Lane SM. 2017. The role of skill in animal contests: a neglected component of fighting ability. Proc R Soc B Biol Sci. 284:20171596.

Cornwallis CK, Uller T. 2010. Towards an evolutionary ecology of sexual traits. Trends Ecol Evol. 25:145–152.

Dennenmoser S, Christy JH. 2013. The design of a beautiful weapon: Compensation for opposing sexual selection on a trait with two functions. Evolution. 67:1181–1188.

Elwood RW, Arnott G. 2012. Understanding how animals fight with Lloyd Morgan's canon. Anim Behav. 84:1095–1102.

Enquist M, Leimar O. 1983. Evolution of fighting behaviour: Decision rules and assessment of relative strength. J Theor Biol. 102:387–410.

Enquist M, Leimar O. 1987. Evolution of fighting behaviour: The effect of variation in resource value. J Theor Biol. 127:187–205.

Fawcett TW, Mowles SL. 2013. Assessments of fighting ability need not be cognitively complex. Anim Behav. 86:e1–e7.

Hammerstein P, Parker GA. 1982. The asymmetric war of attrition. J Theor Biol. 96:647–682.

Hsu Y, Lee SP, Chen MH, Yang SY, Cheng KC. 2008. Switching assessment strategy during a contest: fighting in killifish *Kryptolebias marmoratus*. Anim Behav. 75:1641–1649.

Irschick DJ, Herrel A, Vanhooydonck B, Damme R V. 2007. A functional approach to sexual selection. Funct Ecol. 21:621–626.

Judge KA, Bonanno VL. 2008. Male weaponry in a fighting cricket. PLoS One. 3.

Lailvaux, SP, Irschick DJ. 2006. A functional perspective on sexual selection: insights and future prospects. Anim Behav. 72:263–273.

Lane SM, Briffa M. 2017. The price of attack: rethinking damage costs in animal contests. Anim Behav. 126:23–29.

Maynard-Smith J. 1982. Evolution and the Theory of Games. 1st ed. Cambridge: Cambridge University Press.

McCullough EL. 2014. Mechanical limits to maximum weapon size in a giant rhinoceros beetle. Proc R Soc B Biol Sci. 281:20140696.

Mesterton-Gibbons M, Marden JH, Dugatkin LA. 1996. On wars of attrition without assessment. J Theor Biol. 181:65–83.

Parker GA. 1974. Assessment strategy and the evolution of animal conflicts. J Theor Biol. 47:223–243.

Payne RJH. 1998. Gradually escalating fights and displays: the cumulative assessment model. Anim Behav. 56:651–662.

Payne RJH, Pagel M. 1996. Escalation and time costs in displays of endurance. J Theor Biol. 183:185–193.

Taylor PW, Elwood RW. 2003. The mismeasure of animal contests. Anim Behav. 65:1195–1202.

Vieira MC, Peixoto PEC. 2013. Winners and losers: A meta-analysis of functional determinants of fighting ability in arthropod contests. Funct Ecol. 27:305–313.

Yasuda CI, Koga T. 2016. Importance of weapon size in all stages of male–male contests in the hermit crab *Pagurus minutus*. Behav Ecol Sociobiol.

CHAPTER ONE

How offensive capacity and switching assessment between contest stages can hinder empirical testing of fighting assessment models

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ABSTRACT

Evolutionary game theory provides distinct assessment models to explain how animals decide when to withdraw from a fight. Such models presume different relationships between proxies of contest costs and fighting ability, for instance contest duration and body size, allowing the empirical evaluation of which model better explain the contest behavior of species. However, most studies do not consider two recently raised issues that may affect such relationships: (i) different species may present distinct allometries between offensive and defensive traits, and thus, different abilities to impose damage to the opponent (i.e. offensive capacity), (ii) different contest stages may rely on different forms of assessment (i.e. switching assessment). In this work, we simulated populations that vary in offensive capacities or that switch assessment strategies between stages to evaluate how such features may turn it difficult to distinguish between assessment models with empirical data. Our results demonstrate that depending on the offensive capacity, cumulative assessment contests may generate different relationships between contest duration and body size, which are similar to the expected relationships for pure self-assessment (low offensive capacity), mutual assessment (medium offensive capacity) and opponent-only assessment (high offensive capacity). We also demonstrate that when contests phases follow different assessment strategies, the evaluation of overall contest duration without discriminating the duration of distinct stages leads to the erroneous conclusion that the assessment strategy used in the first phase is the only applied strategy. Our findings shows that inconclusive or contrasting results between contest duration and contest structure, usually found in the literature, may be generated by not accounting for the offensive capacity of species or the possibility of switching assessment between contest stages.

KEYWORDS: Evolutionary game theory, Cumulative assessment, allometry, weaponry, Resource Holding Potential, fight behavior.

INTRODUCTION

Most animal contests resolve when the loser decides to withdraw from the fight (Hardy and Briffa 2013), and evolutionary game theory models suggest different assessment strategies animals may use for this decision (Enquist and Leimar 1983; Enquist and Leimar 1987; Mesterton-Gibbons et al. 1996; Payne and Pagel 1996a; Payne 1998). Many empirical studies have attempted to determine which of these strategies best describe fighting behavior of different species (Arnott and Elwood 2009). To test among such assessment models, researchers evaluate the relationship between proxies of contest costs (e.g. contest duration) and fighting ability (e.g. body size) (Taylor and Elwood 2003). Different assessment strategies present distinct predictions for these relationships (Table 1), but many studies using this approach found evidences supporting more than one assessment model, or did not find evidences for any of these models at all (Jennings et al. 2004; Morrell et al. 2005; Kelly 2006; Prenter et al. 2006; Smallegange et al. 2007; Moore et al. 2008).

Two recently raised problems may be partly responsible for these inconclusive or contrasting results. First, when evaluating injurious contests, most studies consider that traits associated to the ability to impose damage (weaponry) or to endure damage (defenses) are linearly associated to body size (Palaoro and Briffa 2017). This may not be the case for most species, and the relative effect of loser and winner body size on contest duration should be different for species with different allometries (Palaoro and Briffa 2017). Second, the majority of studies do not consider the possibility that different contest phases may follow different assessment strategies (but see Lobregat et al., in preparation

- Chap. 2 of this thesis; Hsu et al. 2008; Yasuda et al. 2012; McGinley et al. 2015). In fights where more than one assessment strategy occurs, the attempt to designate a single strategy to the whole fight may generate inconclusive results as features of different strategies may be present (Hsu et al. 2008). Therefore, the currently accepted empirical predictions of assessment strategies may not fully capture how individuals access fighting ability be affected when we consider these features in the agonistic behavior of species, hindering the correct assignment of assessment strategies to the behavior of species.

Assessment strategy models

Game theory models suggest distinct ways in which rivals assess information about its own or the opponent's fighting ability (Arnott and Elwood 2009). There are four main strategies suggested in the literature:

1) Pure self-assessment: based in models like Energetic War of Attrition (E-WOA) and War of Attrition Without Assessment (WOA-WA), states that contestants' decision to leave the contest occurs when they reach a self-threshold of costs determined by its fighting ability (Mesterton-Gibbons et al. 1996; Payne and Pagel 1996b). Thus, the only determinant of an individual's decision to leave the contest is the information about its own fighting ability.

2) Self-assessment with damage imposition: based in the Cumulative Assessment Model (CAM), states that injuries caused by the opponent accelerate the rate at which individuals reach their cost-threshold (Payne 1998). Stronger opponents are able to impose greater injuries, and so the fighting ability of winners is also expected to influence the losers' decision to retreat.

3) Mutual assessment: based in the Sequential Assessment Model (SAM), states that individuals do not fight according to a cost threshold (Enquist and Leimar 1983), but on information about their relative fighting ability with their rival. According to it,

contestants continuously gather information about the opponent along the contest that ultimately allows the weaker rival to assess its relative inferiority and withdraw from the contest (Enquist and Leimar 1983). As both information about the rivals and about oneself is used in this process, fighting ability of both loser and winner are expected to affect contestants' decision to withdraw.

4) Opponent-Only Assessment (OOA): suggested by some empirical studies which found evidence that the only determinant of an individual's persistence in the contest was the information gathered about their rivals' fighting ability, in such a way that contests take less time to resolve as fighting ability of the winner increases (Elwood and Arnott 2012).

To evaluate which strategy better describes contest, empirical studies most frequently apply the framework proposed by Taylor and Elwood (2003). These authors have suggested that, as assessment strategies differ in the importance of loser or winner fighting ability to the withdraw decision, the relationships between overall contest duration and a measure of fighting ability of losers and winners allow to discriminate what assessment strategy better explain how the decision to leave the fight is taken (Table 1). As mutual and cumulative assessment expect the same relationships, an additional approach is to evaluate contests between size-matched rivals (Taylor and Elwood 2003; Arnott and Elwood 2009; Elwood and Arnott 2012). This analysis states that, as mutual assessment contests are decided based on relative fighting ability of rivals, difference in body size is the ultimate driver of contest duration, and contests between rivals with similar size provide no relationship between contest duration and mean body size of rivals. In counterpart, in cumulative assessment contests, the decision to leave the fight is based on a cost-threshold associated to fighting ability, so that larger rivals have a higher threshold and persist longer. Thus, a positive relationship between contest duration and mean body size is expected.

Table 1. Predictions of assessment strategies for the relationship between contest duration and body size in empirical studies. Based on Taylor & Elwood (2003), Arnott & Elwood (2009) and Elwood & Arnott (2012).

Model	**Effect on contest duration**		
	Loser body size	**Winner body size**	**Mean body size (size-matched contests)**
Pure self-assessment	Positive	Positive, but no significant	Positive
Cumulative assessment	Positive	Negative	Positive
Mutual assessment	Positive	Negative	No relationship
Opponent-only assessment	No relationship	Negative	Negative

Different allometries and injurious contests

Particularly in regard to the predictions of Cumulative Assessment Model (CAM), both the damage imposed by the opponent, as well as the damage taken and the capacity to persist longer (i.e. stamina), are important in the results of a contest (Payne 1998). For most species it is not possible to directly estimate total imposed and received damage, and empirical studies usually simplify this by assuming a linear relationship between indirect measures of fighting ability and the ability to inflict or endure damage (Arnott and Elwood 2009; Vieira and Peixoto 2013; Palaoro and Briffa 2017). However, species may present distinct allometries resulting in different growth rates for offensive and defensive traits, and thus, different investments in damage imposition and damage endurance (Palaoro and Briffa 2017). As Palaoro and Briffa (2017) noticed, this is not considered in most studies, but it may have profound effects on the relationship between contest duration and body size. The same proportional increase in body size for two species with distinct allometries may lead to a different proportional increase in damage imposition and endurance abilities. Thus, we should expect for these species a different relative importance of winner and loser body size on contest duration, and contests following cumulative assessment rules could present different scenarios according to the allometry of a species (Palaoro and Briffa, 2017).

Switching assessment contests

Another scenario not considered in most studies is that different contest phases may follow different assessment strategies. When contests follow a single strategy, assessment models predict that different contest phases may differ only in the intensity of behavioral acts performed along each of them, but the relationship between contest duration and fighting ability should remain the same (Briffa and Elwood 2009). Contrary to this, switching assessment strategies among contest phases may follow predictions from different models, and thus, contests would not present a global assessment strategy (Hsu et al. 2008; Mesterton-Gibbons and Heap 2014). Evidence for this came from recent empirical studies that evaluated the relationship between proxies of fighting ability and duration of each contest phase instead of overall duration (Hsu et al. 2008; Yasuda et al. 2012; McGinley et al. 2015), and theoretical studies have been demonstrating situations in which switching assessment would be beneficial (Mesterton-Gibbons and Heap 2014). As initial phases are always present but not all contests escalate to subsequent phases, it is expected that initial phases would present a higher influence on total contest duration. Thus, if each phase follows different assessment strategies, the evaluation of overall contest duration alone may lead to the conclusion that the whole fight is explained by the assessment strategy employed only at the beginning of the contest.

Here, we simulated alternative assessment strategies following a similar approach to Taylor & Elwood (2003), and tested their predictions, considering body size as proxy for fighting ability. We arranged pairings between individuals derived from simulated populations and for each pairing we established contests with the rules employed by self-assessment, cumulative assessment, mutual assessment and opponent-only assessment strategies. For cumulative assessment contests, however, we simulated three scenarios with different offensive capacities of individuals. Then, we evaluated the relationship between contest duration and body size of contestants and compared the results for different assessment strategies. With such approach, we tested the hypothesis that cumulative assessment contests may present different relationships between contest duration and body size depending on the offensive capacity (allometry between weapon and body size) of the contestants. In the second approach, we set different assessment strategies for different contest phases and evaluated the relationship between overall contest duration and duration of each phase with body size of contestants. With this, we tested the hypothesis that in fights where two contest phases follow different assessment strategies, the evaluation of overall contest duration alone prevents the correct assignment of the second contest stage.

SIMULATION PROCEDURE

We simulated 1000 populations of 200 individuals each, with body size normally distributed around a mean of 30 cm and a standard deviation of 6 cm. For each population, we sorted 100 pairings that we used as contests, and considered winners as the individuals with higher fighting ability in each pairing. Body size is usually a good predictor of fighting ability (Arnott and Elwood 2009; Vieira and Peixoto 2013), but it does not perfectly determine fighting ability, as other several factors may influence fighting ability of individuals like experience, age, physiological attributes and so on (Vieira and Peixoto 2013). Thus, we simulated actual fighting ability by adding a standard deviation of four to values of body size. In this way, most frequently larger individuals had higher fighting ability, but sometimes smaller individuals were better fighters. To evaluate size-matched contests empirical predictions, we determined size-matched contests by creating a similarity threshold of 10% of the total variation in body size for each population, and assigned size-matched contests with a difference in body size smaller than this threshold. Our simulation innovates, compared to previous studies (Taylor and Elwood 2003; Morrell et al. 2005; Fawcett and Mowles 2013), by simulating multiple populations, allowing the evaluation of the variation within and between populations, which enabled endorsing not only the resulting correlations, but also the frequency of statistical results.

Hypothesis 1: Different offensive capacities

To evaluate how different offensive capacities affect the relationship between body size and contest duration, and to compare these results with other assessment strategies, we simulated pairings within each of the 1000 simulated populations, to determine contest duration under different assessment strategy rules. For each pairing, we calculated its contest duration under the rules of pure self-assessment, cumulative assessment, mutual assessment and opponent-only assessment, as presented in Table 2. In cumulative assessment contests, we calculated damage caused by the opponent as:

$$Damage = X^a \qquad (1)$$

where X is individual body size and a is a scaling component representing offensive capacity. Here, we assumed that damage is determined by size of damage-imposing structures (weaponry). Equation 1 represents the allometric nature of the relationship between overall body growth and weaponry growth (Huxley 1924). Parameter value $a < 1$ stands for cases where weapons grow at a slower rate than body size, $a > 1$ represents the opposite, and $a = 1$ represent equal growth rates for weapons and body size. We calculated damage in three scenarios of cumulative assessment: low offensive capacity ($a = 0.8$), medium offensive capacity ($a = 1$) and high offensive capacity ($a = 1.2$).

Hypothesis 2: Switching assessment strategies

We tested if the evaluation of overall contest duration alone may lead to the conclusion that the first stage strategy is applied along the entire contest, hindering the detection of another strategy applied in the escalated stage. For this, we simulated fights with two contest phases in a scenario where the first phase follows mutual assessment and the second phase follows pure self-assessment. As not all contests are expected to escalate to final phases (Enquist and Leimar 1983), we determined rules of contest escalation. Mutual assessment states that contests escalate to more intense phases when rivals have similar fighting abilities, as initial phases do not provide accurate information to allow the detection of relative fighting in such cases (Enquist and Leimar 1983). Thus, we adjusted the probability of escalation as a function of the difference in fighting ability between contestants using the following equation:

$$Probability\ of\ escalation = \frac{ST}{1+e^{(b.x)}+\frac{ST}{2}} \tag{2}$$

where, ST is the similarity threshold for that population, b is the slope of the effect of difference in body size on the probability (set as -0.4), and x is the difference in body size. This equation follows the probability function formulae of a binomial distribution, which is used to calculate the probability of a given event occurs or not (in this case, escalation to the second phase) according to a given variable (in this case, difference in body size between contestants).

Table 2. Contest duration under different assessment strategies. For each pairing, we calculated contest duration following the general assumption of losers' decision to withdraw indicated by each assessment strategy. Intercept of 50 was added to the equations of cumulative assessment, mutual assessment and opponent-only assessment, in order to avoid negative values, but the model assumption remains the same. Negative values of contest duration were not impossible, for both cumulative and opponent-only assessment, but were extremely rare, and could be interpreted as corresponding to duration = zero. We

interpret zero duration as “the fight would not occur”, which is biologically illogical, as far as assessment must take at least some seconds to occur, so as to resolve the fight.

Assessment strategy	**Contest duration equation**	**General assumption**
Pure self-assessment	Duration = LosRHP	Contest duration is solely determined by the loser’s cost threshold
Cumulative assessment	Duration = 50+LosRHP– WinBS^{OC}	Contest duration is determined by the loser’s cost threshold, which is negatively affected by winner’s injuries
Mutual assessment	Duration = 50 – DiffRHP	Contest duration is negatively determined by the relative fighting ability between rivals
Opponent-Only Assessment	Duration = 50 – WinRHP	Contest duration is solely determined by information about winner’s fighting ability

LosRHP = loser fighting ability; WinBS = winner body size; OC = offensive capacity; DiffRHP = difference in fighting ability between rivals; WinRHP = winner fighting ability

After this, we simulated for each contest a binary outcome (1 = escalation, 0 = no escalation) following a binomial distribution with the probability function calculated above. Finally, we calculated the duration of each phase following their assessment strategy rules (see Table 2), and considered overall contest duration as the sum of the duration of these phases in escalated contests or the duration of the first phase in non-escalated contests.

Relationship between contest duration and body size

For each population under each simulated scenario of assessment strategy, we adjusted linear models where we defined contest duration as the response variable and loser and winner body as predictor variables in the same model (multiple regression) or in separate models (simple regressions). We also evaluated the relationship between contest duration and mean body size for size-matched contests of each population. For each population we estimated the slopes of the predictor variables, the standard error of these slopes and their p-values, in order to compare the results from different assessment strategies (Hypothesis 1) or different contest phases (Hypothesis 2). Since each population had its own results for the regression analyses, we calculated for each assessment strategy scenario the 95% more frequent values found for the slopes, the total variation in the standard error of the slopes and the total number of populations in which the predictors p-values were significant (<0.05). All simulations and statistical analyses were performed with R (R Core Team 2018).

RESULTS FOR CUMULATIVE ASSESSMENT CONTESTS WITH DIFFERENT OFFENSIVE CAPACITIES

Randomly matched contests

We present the relationships between fight duration and body size of one of the 1000 simulated populations, under the different assessment strategies: cumulative assessment with low offensive capacity and pure self-assessment (Fig. 1), cumulative assessment with high offensive capacity and opponent-only assessment (Fig. 2), cumulative assessment with medium offensive capacity and mutual assessment (Fig. 3). As we expected, the relationship between contest duration and body size in cumulative assessment contests varied with offensive capacities. As offensive capacities increased, the slope values of winner body size in multiple (Table 3) and simple (Table 4) regression analyses were progressively greater in absolute values. This changed the relative effects of loser and winner body size on contest duration among offensive capacities. In contests where the offensive capacity was small (Figure 1), loser body size was the main predictor of contest duration. The effect of winner body size was smaller than loser's body size, and for many populations it was non-significant (141 populations in multiple regression and 952 populations in simple regression). Thus, cumulative assessment contests where individuals are not able to impose great injuries to their opponents, may be wrongly assigned to pure self-assessment, if empirical predictions of the literature are strictly followed.

On the other hand, contests with high offensive capacity showed a higher effect of winner body size than loser body size on contest duration (Figure 2), depicted in the higher absolute values of winner, compared to loser, body size slopes. However, multiple and simple regressions generated different results for the significance of loser body size, leading to disparate conclusions for this scenario (Table 6). Multiple regression supported literature predictions for cumulative assessment: loser body size had a positive effect, and winner body size had a negative effect on contest duration (Taylor and Elwood 2003; Arnott and Elwood 2009). In counterpart, simple regression provided results which are considered indicative of opponent-only assessment predictions in the literature (Arnott and Elwood 2009; Elwood and Arnott 2012): loser body size had no relationship with contest duration for 84.3% of all simulated populations, while winner body size had always a significant negative relationship. Therefore, our results demonstrate that simple regression is not adequate to distinguish among alternative contest strategies. When multiple explanatory variables affect the same response variable, it is not correct to build models that exclude one of them, because the relation of the response with the explanatory variable in the model may depend on the effect of the other variable (Crawley 2013). Following these rationale, multiple regression should be adopted preferentially to simple regression.

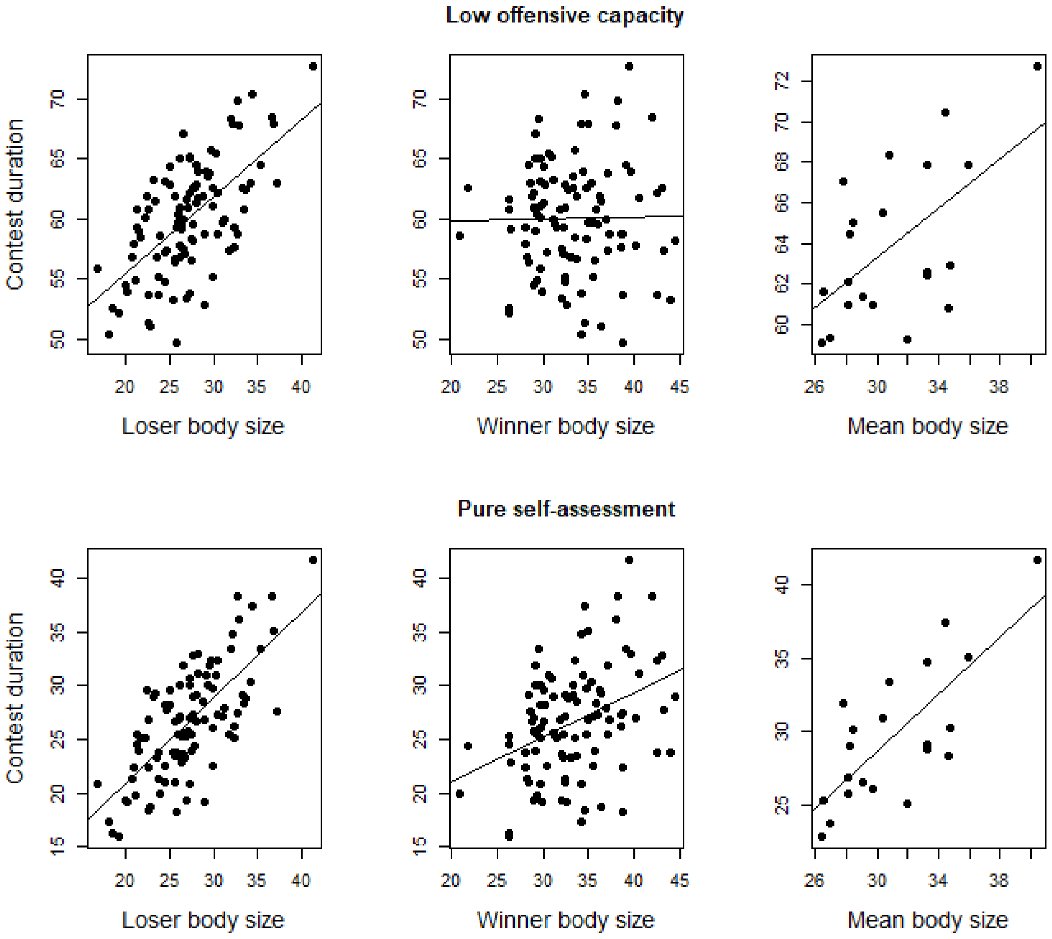

Figure 1. Results of one simulated population for the relationships between contest duration and body size under cumulative assessment with low offensive capacity and pure self-assessment. Mean body size relationship was determined only for size-matched contests. Plotted regression lines were calculated with simple regression.

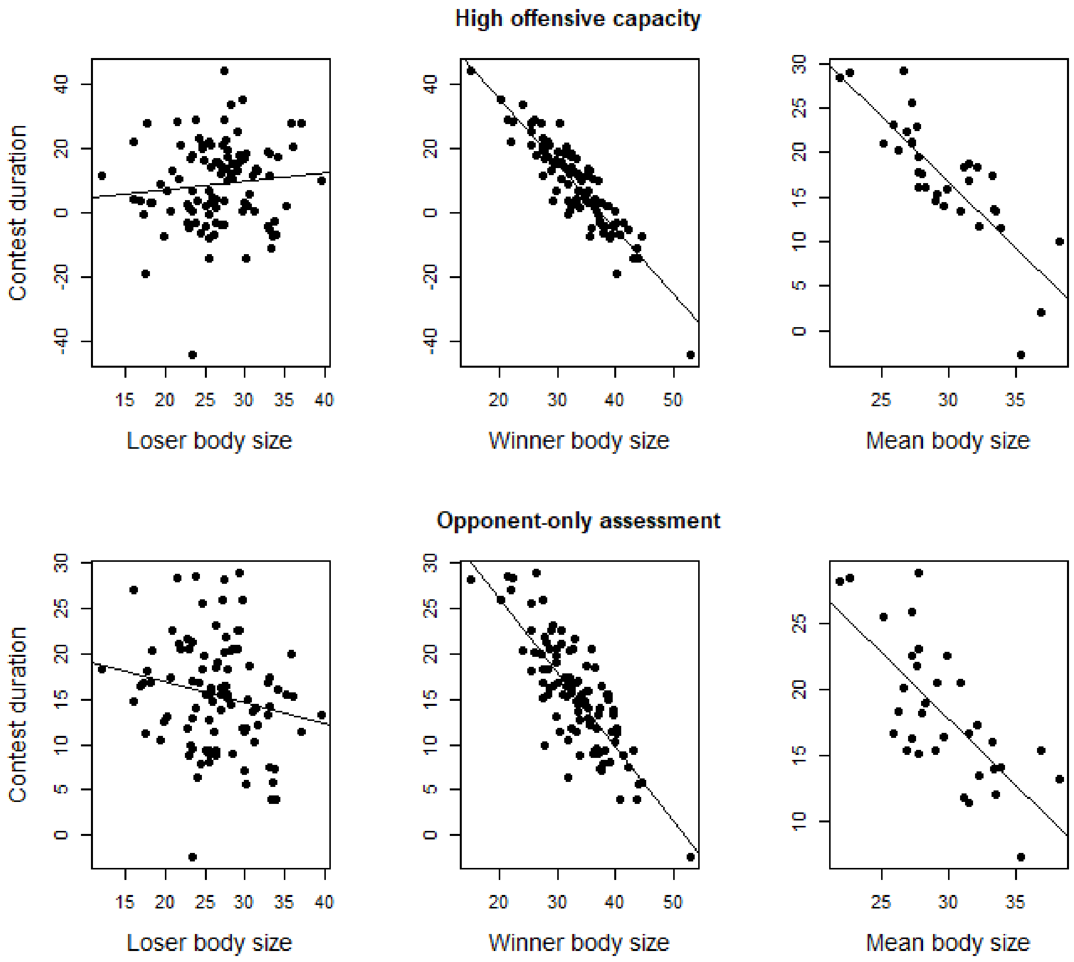

Figure 2. Results of one simulated population for the relationships between contest duration and body size under cumulative assessment with high offensive capacity and opponent only assessment. Mean body size relationship was determined only for size-matched contests. Plotted regression lines were calculated with simple regression.

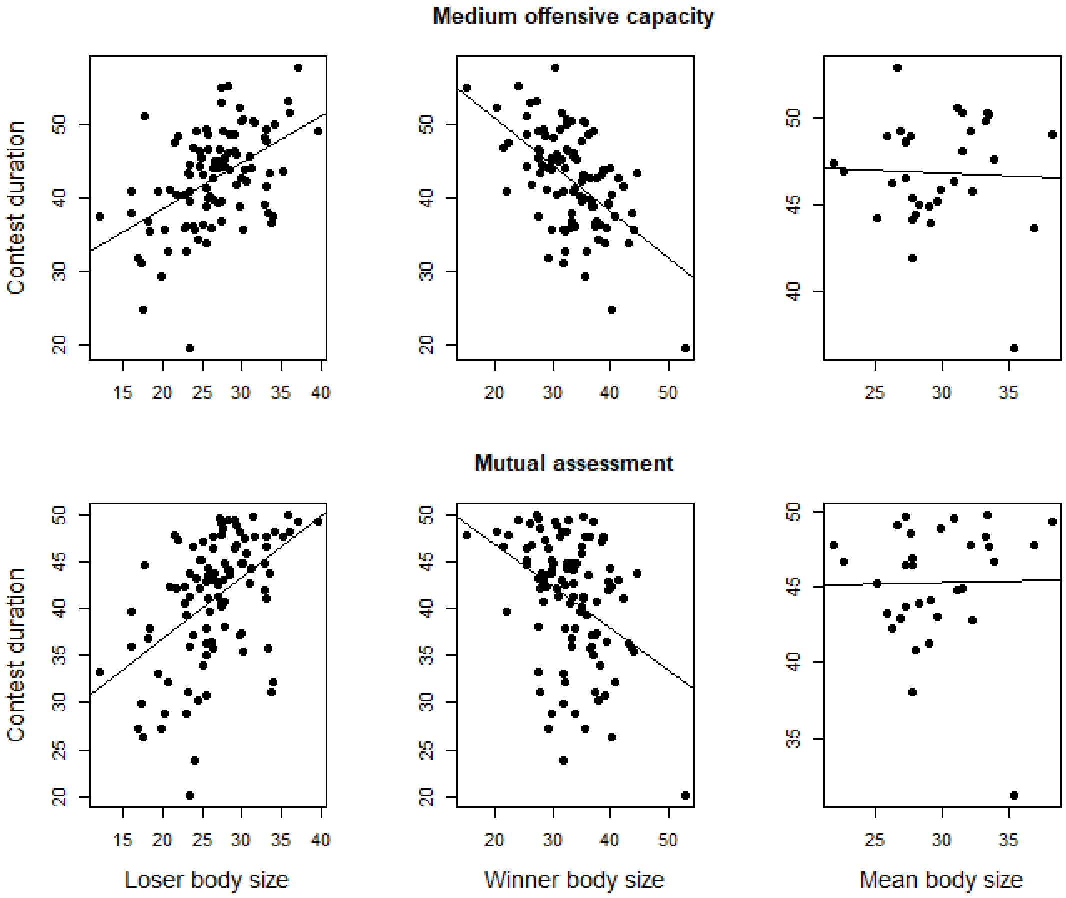

Figure 3. Results of one simulated population for the relationships between contest duration and body size under cumulative assessment with high offensive capacity and opponent only assessment. Mean body size relationship was determined only for size-matched contests. Plotted regression lines were calculated with simple regression.

Notwithstanding their shortcomings, simple regression may provide usefull information. Our simulations provide a simplified situation, insofar as they do not include additional mechanisms that may interfere on contest duration, like motivation and fighting experience. Such additional features could inflate error for the relationship between loser body size and contest duration, reducing chances of significant relationship. Given this, and the highly disparate results between approaches, we should not discard the indication from our simple regressions that scenarios interpreted as opponent-only assessment may result from cumulative assessment with high offensive capacity.

Table 3. Results from multiple regression analyses between contest duration and body size of losers and winners. For each parameter, we estimated its total variation along the 1000 simulated populations and the variation for the 95% more frequent values. For the loser and winner body size slopes we also estimated the variation in standard error and the number of populations that demonstrated a significant p-value for that parameter.

Model	Parameter	Variation (95% more frequent)	Standard error of the slope	P-values <0.05
Pure self-assessment	Loser slope	0.59 to 1.03 (0.68 to 0.97)	0.05 to 0.09	1000
	Winner slope	-0.02 to 0.4 (0.03 to 0.32)	0.05 to 0.09	673
Cumulative assessment with low offensive capacity	Loser slope	0.59 to 1.03 (0.68 to 0.97)	0.05 to 0.09	1000
	Winner slope	-0.42 to -0.001 (-0.36 to -0.08)	0.13 to 0.24	859
Cumulative assessment with medium offensive capacity	Loser slope	0.59 to 1.03 (0.68 to 0.97)	0.05 to 0.09	1000
	Winner slope	-1.02 to -0.61 (-0.96 to -0.68)	0.05 to 0.09	1000
Cumulative assessment with high offensive capacity	Loser slope	0.6 to 1.04 (0.68 to 0.98)	0.05 to 0.09	1000
	Winner slope	-2.43 to -2.01 (-2.38 to -2.09)	0.02 to 0.04	1000
Mutual assessment	Loser slope	0.38 to 1.01 (0.45 to 0.84)	0.06 to 0.13	1000
	Winner slope	-0.96 to -0.35 (-0.84 to -0.45)	0.06 to 0.13	1000
Opponent-only assessment	Loser slope	-0.44 to 0.1 (-0.32 to -0.03)	0.05 to 0.1	665
	Winner slope	-1.06 to -0.6 (-0.97 to -0.68)	0.05 to 0.1	1000

Table 4. Results from simple regression analyses between contest duration and body size of losers and winners. For each parameter, we estimated its total variation along the 1000 simulated populations and the variation for the 95% more frequent values. For the loser and winner body size slopes we also estimated the variation in standard error and the number of populations that demonstrated a significant p-value for that parameter.

Model	Parameter	Variation (95% more frequent)	Standard error of the slope	P-values <0.05
Pure self-assessment	Loser slope	0.66 to 1.09 (0.73 to 1.01)	0.05 to 0.09	1000
	Winner slope	0.06 to 0.61 (0.03 to 0.32)	0.08 to 0.15	965
Cumulative assessment with low offensive capacity	Loser slope	0.55 to 0.99 (0.68 to 0.97)	0.05 to 0.09	1000
	Winner slope	-0.38 to 0.35 (-0.19 to -0.21)	0.08 to 0.15	48
Cumulative assessment with medium offensive capacity	Loser slope	0.26 to 0.93 (0.38 to 0.82)	0.08 to 0.15	1000
	Winner slope	-0.94 to -0.26 (-0.96 to -0.68)	0.08 to 0.15	1000
Cumulative assessment with high offensive capacity	Loser slope	-0.57 to 1.003 (-0.27 to 0.68)	0.17 to 0.34	157
	Winner slope	-2.37 to -1.67 (-2.22 to -1.8)	0.08 to 0.15	1000
Mutual assessment	Loser slope	0.1 to 0.91 (0.23 to 0.71)	0.08 to 0.15	983
	Winner slope	-0.82 to -0.1 (-0.69 to -0.26)	0.08 to 0.15	987
Opponent-only assessment	Loser slope	-0.8 to 0.02 (-0.62 to -0.18)	0.08 to 0.15	953
	Winner slope	-1.12 to -0.63 (-1.01 to -0.74)	0.05 to 0.09	1000

In medium offensive capacity, where weaponry and body size increase at an equal rate, we found opposite effects of similar absolute values for the effect of loser and winner body size on contest duration, for both simple and multiple regression approaches. These results are similar to what is expected on the literature for mutual assessment (Taylor and Elwood 2003). Thus, to distinguish cumulative from mutual assessment when slopes of loser and winner body size are similar, we should analyze the patterns of escalation and de-escalation in intensity between and within phases (Briffa and Elwood 2009): while cumulative assessment allows for de-escalation within phases, mutual assessment predicts escalation among phases and homogenous contest intensity within phases.

Size-matched contests

Changing offensive capacities in cumulative assessment, shows even larger difficulties in distinguishing it from other assessment strategies for size-matched contests (Table 5). Some theoretical studies suggest that for size-matched contests, the relationship between contest duration and mean body size should be positive in cumulative assessment contests (Arnott and Elwood 2009; Elwood and Arnott 2012). However, our results demonstrate that such relationship should be only expected for small offensive capacity. When weaponry increases linearly with body sizes (medium offensive capacity), there was no relationship between contest duration and mean body size, which is the same result expected for mutual assessment (Elwood and Arnott 2012; Fawcett and Mowles 2013). When offensive capacity is high, there was a negative relationship between contest duration and mean body size, fitting the predictions of opponent-only assessment. Thus, unless the relationship between body size and damage imposition is clearly known for the studied species, the analysis of size-matched contests is not able to discriminate between any assessment strategy.

Table 5. Results from simple regression analyses between contest duration and mean body size of individuals in size-matched contests. We estimated the total variation for the slope along the 1000 simulated populations and the variation found for the 95% more frequent values. We also estimated the variation in standard error and the number of populations that demonstrated a significant p-value.

Model	Variation in slope (95% more frequent)	Standard error of the slope	P-values <0.05
Pure self-assessment	0.49 to 1.55 (0.68 to 1.28)	0.08 to 0.36	1000
Cumulative assessment with low offensive capacity	0.08 to 1.15 (0.28 to 0.88)	0.08 to 0.36	920
Cumulative assessment with medium offensive capacity	-0.54 to 0.58 (-0.33 to -0.29)	0.09 to 0.37	50
Cumulative assessment with high offensive capacity	-1.98 to -0.61 (-1.76 to -1.01)	0.1 to 0.4	999
Mutual assessment	-0.63 to 0.54 (-0.34 to 0.32)	0.09 to 0.15	55
Opponent-only assessment	-1.55 to -0.44 (-1.3 to -0.69)	0.08 to 0.32	999

RESULTS FOR SWITCHING ASSESSMENT CONTESTS

As we expected, when we simulated a first phase as mutual assessment and a second phase as pure self-assessment, the effect of loser and winner body size on overall contest duration were similar to the patterns of first phase duration: duration increased with loser and decreased with winner body size (Table 6). In contrast, analysing the second phase separately, the results supported the actual simulation rule of pure self-assessment. This shows that when contest phases are not considered in the analysis of duration and body size, we may hide important information of what assessment strategy a species is employing in the escalated phases. This probably occurs due to the higher influence of the first phase on overall duration, since all contests must pass through the first phase, but not all contests escalate to the second phase. This was evident in our analyses, where mean number of contests that escalated to second phase in each population was 28, out of 100 (Supplementary Material). The analysis of overall duration alone lead to the wrong illusion that the assessment strategy employed in the first phase occurs throughout the entire fight. When not considering eventual changes in assessment strategy among phases, contrasting features may be wrongly interpreted as ambivalent evidences.

DISCUSSION

Our simulations uphold the importance of considering the relationship between the ability to impose damage and the ability to endure damage in empirical evaluation of injurious contests (Palaoro and Briffa 2017). Here, we simulated alternative scenarios for the offensive capacity and maintained damage endurance (body size) fixed, but similar relationships would be expected if we controlled offensive capacity and altered the ability to endure damage (see Palaoro & Briffa, 2017). Our goal was to demonstrate that different weights for damage imposition relative to damage endurance, can greatly affect cumulative assessment predictions of contest duration relationship with loser and winner body size. Thus, injurious contests with cumulative assessment could be erroneously assigned to other assessment strategies (Table 7). For example, in *Bradypodion pumilum* chameleon contests, the relationship between contest duration and body size of losers and winners suggests pure self-assessment as the most representative strategy (Stuart-Fox 2006). However, the repertoire of agonistic behaviors applied along the fight include actions that are expected to cause some injury like mouth-wrestling and biting, and the analysis of the patterns of escalation fits cumulative assessment predictions (Stuart-Fox, 2006). Thus, it is possible that damage imposition has a relatively minor role than individual persistence in these contests, but individual persistence alone does not explain how losers' threshold of costs is achieved. Many other species support pure self-assessment predictions of contest duration but present injury imposition behaviors, like parasitoid wasps (Tsai et al. 2014), jumping spiders (Taylor et al. 2001; Elias et al. 2008), orb-weaving spiders (Bridge et al. 2000) and amphipods (Prenter et al. 2006). Therefore, it would be valuable to investigate the allometric relationship between body and weaponry size or, even better, some direct measure of damage imposition, in order to evaluate if their offensive capacities are indeed low.

Table 6. Results from multiple regression analyses between body size of losers and winners and measures of duration in contests where the first phase follows mutual assessment and the second phase follows pure self-assessment. We estimated the total variation for the slope of losers and winners body size along the 1000 simulated populations and the variation found for the 95% more frequent values. We also estimated the variation in standard error and the number of populations that demonstrated a significant p-value for each slope.

Measure of duration	**Parameter**	**Variation (95% more frequent)**	**Standard error of the slope**	**P-values <0.05**
Overall duration	Loser slope	0.45 to 1.65 (0.71 to 1.44)	0.14 to 0.25	1000
	Winner slope	-1.44 to -0.42 (-1.26 to -0.59)	0.13 to 0.26	998
First phase duration (mutual assessment)	Loser slope	0.38 to 1.01 (0.45 to 0.84)	0.06 to 0.13	1000
	Winner slope	-0.96 to -0.35 (-0.84 to -0.46)	0.06 to 0.13	1000
Second phase duration (pure self-assessment)	Loser slope	0.03 to 0.55 (0.16 to 0.46)	0.04 to 0.18	967
	Winner slope	-0.04 to 0.41 (0.05 to 0.34)	0.04 to 0.16	776

The same reasoning could be applied to cumulative assessment contests with high offensive capacity. Our results provide evidences that when damage is very high, cumulative assessment contest duration may be negatively affected solely by winner body size. This suggests an alternative explanation to opponent-only assessment, if fights involve damage exchange. It could be the case of contests between *Neolamprologus pulcher* cichlid fishes that fight over dominance hierarchy over social groups (Reddon et al. 2011). In this species, contest duration has no relationship with loser body size and presents a negative relationship with winner body size. In addition, patterns of intensity escalation and de-escalation fit cumulative assessment predictions: fights begin with agonistic displays for some seconds, and then escalate to injury imposition with a vast repertoire of agonistic actions, with intermittent de-escalation to display behavior (Sopinka et al. 2009; Reddon et al. 2011). However, there is no enlarged structure specialized in injury imposition in this species, and a possible explanation is that damage endurance could be low in these fishes, which would induce similar results of a high offensive capacity.

Most empirical studies that suggest opponent-only assessment are based on display behaviors, not injurious contests (Rillich et al. 2007; Prenter et al. 2008; Arnott and Elwood 2010; Jennings et al. 2012). Thus, although our reasoning that opponent-only assessment is just a by-product of cumulative assessment possibilities seems correct, empirical support for this is scarce. Nevertheless, a result similar to that expected by cumulative assessment, could be generated by mutual assessment contests where information about the opponent has a greater influence in the decision to leave the contest than own information. The mathematical reasoning would be the same: if the effect of winner fighting ability on contest duration is very strong, a positive significant effect of loser body size on contest duration is not required to the statement that loser fighting ability is also used in the decision to withdraw. The theoretical reasoning would also be upheld. Theory states that display behaviors minimize fight-associated costs by increasing the chances of fight resolution before the fight escalate (Hurd 1997; Payne and Pagel 1997; Zahavi and Zahavi 1999; Vehrencamp 2000). In species with high offensive capacities, the potential damage costs of fight escalation could be very expensive, and information gathered with opponent displays could overwhelm the importance of own information in the decision to leave the contest.

Table 7. Summary of the possible relationships between contest duration and body size of losers and winners in different scenarios of offensive capacity in cumulative assessment contests. Multiple and simple regression relationships are shown. We show the upheld models by the relationships found in each scenario, according to the literature.

Scenario	Regression method	Effect on contest duration		Corroborated models
		Loser size	**Winner size**	
Low offensive capacity	Multiple	Positive*	Positive n.s.	WOA
	Single	Positive*	No effect	WOA
Medium offensive capacity	Multiple	Positive*	Negative*	SAM and CAM
	Single	Positive*	Negative*	SAM and CAM
High offensive capacity	Multiple	Positive*	Negative*	CAM
	Single	No effect	Negative*	OOA

WOA: pure self-assessment; CAM: cumulative assessment; SAM: mutual assessment; OOA: opponent-only assessment; * = more than 950 populations showed a significant relationship.

Our results also demonstrate that the evaluation of overall contest duration without discriminating contest phases hinder the detection of switching assessment between phases. In our simulations, we considered a scenario where fights changes from mutual to pure self-assessment, but other scenarios probably result in the same conclusion. The key point here is that most fights do not progress to escalated phases, and the contribution of such phases to overall contest duration may be highly hampered by the duration of initial phases. Therefore, in fight behaviors where escalated phases are not frequent, the possibility of switching assessment between phases should be considered in order to minimize a possible incorrect conclusion of how individuals assess fighting ability throughout the entire contest.

The first work to suggest that contest phases may follow different assessment strategies was Morrel et al. (2005) with fiddler crabs, and this possibility was explicitly tested for the first time by Hsu et al. (2008) in killifish. Since then, however, few empirical studies considered switching assessment between phases (Yasuda et al. 2012; McGinley et al. 2015), and a great part of this discussion was restricted to theoretical studies (Arnott & Elwood 2009; Elwood & Arnott 2012; Fawcett & Mowles 2013; Mesterton-Gibbons and Heap 2014). As most species present more than one behavioral phase, we argue that switching assessment may occur in a broader range of species than is currently known, and new empirical studies should consider this possibility to help understand how general this scenario might be.

CONCLUSION

Our work demonstrates that different offensive capacities lead to different results for the relationship between contest duration and body size of contestants in cumulative assessment contests. Thus, fights following cumulative assessment may present results that are also expected by other assessment strategies, hindering the correct assignment of which strategy underpins contest behavior. To avoid this problem, we suggest that future studies empirically evaluating injurious contests should take a closer look at the offensive capacity of individuals, either by investigating the allometry of damage imposition structures or by directly evaluating the damage individuals impose and its relationship with body size.

We also show that the evaluation of overall contest duration prevents the detection of the changes in assessment strategy among phases. Since, most studies do not discriminate duration of different contest phases, it is possible that switching assessment is more general than what it is shown in the literature. Thus, we suggest that future studies evaluating the contest behavior of species that present two or more behavioral phases should evaluate not only overall contest duration, but also the duration of different phases.

REFERENCES

Arnott G, Elwood RW. 2009. Assessment of fighting ability in animal contests. Anim Behav. 77:991–1004.

Arnott G, Elwood RW. 2010. Startle durations reveal visual assessment abilities during contests between convict cichlids. Behav Processes. 84:750–756.

Bridge AP, Elwood RW, Dick JT. 2000. Imperfect assessment and limited information preclude optimal strategies in male-male fights in the orb-weaving spider *Metellina mengei*. Proc R Soc B Biol Sci. 267:273–279.

Briffa M, Elwood RW. 2009. Difficulties remain in distinguishing between mutual and self-assessment in animal contests. Anim Behav. 77:759–762.

Crawley MJ. 2013. The R book. 2nd ed. Chichester: Wiley.

Elias DO, Kasumovic MM, Punzalan D, Andrade MCB, Mason AC. 2008. Assessment during aggressive contests between male jumping spiders. Anim Behav. 76:901–910.

Elwood RW, Arnott G. 2012. Understanding how animals fight with Lloyd Morgan's canon. Anim Behav. 84:1095–1102.

Enquist M, Leimar O. 1983. Evolution of fighting behaviour: Decision rules and assessment of relative strength. J Theor Biol. 102:387–410.

Enquist M, Leimar O. 1987. Evolution of fighting behaviour: The effect of variation in resource value. J Theor Biol. 127:187–205.

Fawcett TW, Mowles SL. 2013. Assessments of fighting ability need not be cognitively complex. Anim Behav. 86:e1–e7.

Hardy ICW, Briffa M. 2013. Animal contests. New York: Cambridge University Press.

Hsu Y, Lee SP, Chen MH, Yang SY, Cheng KC. 2008. Switching assessment strategy during a contest: fighting in killifish *Kryptolebias marmoratus*. Anim Behav. 75:1641–1649.

Hurd PL. 1997. Is signalling of fighting ability costlier for weaker individuals? J Theor Biol. 184:83–88.

Huxley JS. 1924. Constant differential growth-ratios and their significance. Nature. 115:895–896.

Jennings DJ, Elwood RW, Carlin CM, Hayden TJ, Gammell MP. 2012. Vocal rate as an assessment process during fallow deer contests. Behav Processes. 91:152–158.

Jennings DJ, Gammell MP, Carlin CM, Hayden TJ. 2004. Effect of body weight, antler length, resource value and experience on fight duration and intensity in fallow deer. Anim Behav. 68:213–221.

Kelly CD. 2006. Fighting for harems: assessment strategies during male-male contests in the sexually dimorphic Wellington tree weta. Anim Behav. 72:727–736.

McGinley RH, Prenter J, Taylor PW. 2015. Assessment strategies and decision making in male-male contests of *Servaea incana* jumping spiders. Anim Behav. 101:89–95.

Mesterton-Gibbons M, Heap SM. 2014. Variation between self- and mutual assessment in animal contests. Am Nat. 183:199–213.

Mesterton-Gibbons M, Marden JH, Dugatkin LA. 1996. On wars of attrition without assessment. J Theor Biol. 181:65–83.

Moore JC, Obbard DJ, Reuter C, West SA, Cook JM. 2008. Fighting strategies in two species of fig wasp. Anim Behav. 76:315–322.

Morrell LJ, Backwell PRY, Metcalfe NB. 2005. Fighting in fiddler crabs *Uca mjoebergi*: What determines duration? Anim Behav. 70:653–662.

Palaoro A V, Briffa M. 2017. Weaponry and defenses in fighting animals: How allometry can alter predictions from contest theory. Behav Ecol. 28:328–336.

Parker GA. 1974. Assessment strategy and the evolution of animal conflicts. J Theor Biol. 47:223–243.

Payne RJH. 1998. Gradually escalating fights and displays: the cumulative assessment model. Anim Behav. 56:651–662.

Payne RJH, Pagel M. 1996a. When is false modesty a false economy? An optimality model of escalating signals. Proc R Soc B Biol Sci. 263:1545–1550.

Payne RJH, Pagel M. 1996b. Escalation and time costs in displays of endurance. J Theor Biol. 183:185–193.

Payne RJH, Pagel M. 1997. Why do animals repeat displays? Anim Behav. 54:109–119.

Prenter J, Elwood RW, Taylor PW. 2006. Self-assessment by males during energetically costly contests over precopula females in amphipods. Anim Behav. 72:861–868.

Prenter J, Taylor PW, Elwood RW. 2008. Large body size for winning and large swords for winning quickly in swordtail males, *Xiphophorus helleri*. Anim Behav. 75:1981–1987.

Reddon AR, Voisin MR, Menon N, Marsh-Rollo SE, Wong MYL, Balshine S. 2011. Rules of engagement for resource contests in a social fish. Anim Behav. 82:93–99.

Rillich J, Schildberger K, Stevenson PA. 2007. Assessment strategy of fighting crickets revealed by manipulating information exchange. Anim Behav. 74:823–836.

Smallegange IM, Sabelis MW, van der Meer J. 2007. Assessment games in shore crab fights. J Exp Mar Bio Ecol. 351:255–266.

Sopinka NM, Fitzpatrick JL, Desjardins JK, Stiver KA, Marsh-Rollo SE, Balshine S. 2009. Liver size reveals social status in the African cichlid *Neolamprologus pulcher*. J Fish Biol. 75:1–16.

Stuart-Fox D. 2006. Testing game theory models: fighting ability and decision rules in chameleon contests. Proc R Soc B Biol Sci. 273:1555–1561.

Taylor PW, Elwood RW. 2003. The mismeasure of animal contests. Anim Behav. 65:1195–1202. doi:10.1006/anbe.2003.2169.

Taylor PW, Hasson O, Clark DL. 2001. Initiation and resolution of jumping spider contests: Roles for size, proximity, and early detection of rivals. Behav Ecol Sociobiol. 50:403–413.

Tsai YJJ, Barrows EM, Weiss MR. 2014. Pure Self-Assessment of Size During Male-Male Contests in the Parasitoid Wasp *Nasonia vitripennis*. Ethology. 120:816–824.

Vehrencamp, S. L. (2000). Handicap, index, and conventional signal elements of bird song. In: Espmark, Y., Amundsen, T., Rosenqvist, G. (eds). Animal Signals: Signalling and Signal Design in Animal Communication, 277–300.

Vieira MC, Peixoto PEC. 2013. Winners and losers: A meta-analysis of functional determinants of fighting ability in arthropod contests. Funct Ecol. 27:305–313.

Yasuda C, Takeshita F, Wada S. 2012. Assessment strategy in male-male contests of the hermit crab *Pagurus middendorffii*. Anim Behav. 84:385–390.

Zahavi A, Zahavi A. 1999. The handicap principle: A missing piece of Darwin's puzzle. New York: Oxford University Press.

CHAPTER TWO

Assessment strategy changes along contests in a Neotropical cricket

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ABSTRACT

Recognizing how animals decide when to withdraw from their fight is central to understand the evolution of fighting behavior. Game theory models suggest two major types of decision criteria: 1) self-assessment where individuals withdraw when they achieve a threshold of costs associated to its own fighting ability, which may or may not be affected by injuries, or 2) mutual assessment where the decision is based on information about relative fighting ability between opponents. Many empirical studies assumed that rivals use one single strategy throughout the whole fight, but recent studies suggest that individuals may change their assessment strategy along contest phases. In the present work, we addressed this issue by evaluating if males of *Melanotes ornata* crickets switch their assessment strategy along their contests. We used hind femur length as a proxy of fighting ability, as it was the best attribute explaining contest outcome. Overall fight duration was positively associated to loser femur length and negatively associated to winner femur length, while the probability of escalation to physical aggression was negatively related to femur length difference between opponents. However, when analyzing duration of different contest phases, such relationships held only at the first phase of the fight, while no relationship between fighting ability and duration was found for escalated phases. These results suggest that *M. ornata* males present mutual assessment in the initial phase of the contest, but switch their assessment strategy when fights escalate, reinforcing that a single strategy may not correctly explain how contests are settled.

KEYWORDS: Evolutionary game theory, switching assessment, aggressive behavior, animal contest, fighting behavior, Grylloidea, Phalangopsidae.

INTRODUCTION

Animals of many species often engage in physical contests for limited resources as potential mates or territories (Hardy and Briffa 2013). In such contests, the winner is usually the individual with higher fighting ability (which is also called Resource Holding Potential, Parker 1974). Such individuals possess characteristics that allow them to pay higher costs to stay in the fight and/or inflict higher costs to the opponent (Maynard-Smith 1982; Maynard-Smith and Parker 1976; Parker 1974; Payne 1998). However, while morpho-physiological characteristics associated to fighting ability are easily identified (Vieira and Peixoto 2013), their role in determining when each rival should withdraw the contest are much debated (Arnott and Elwood 2009; Briffa and Elwood 2009; Elwood and Arnott 2012; Fawcett and Mowles 2013; Taylor and Elwood 2003). The main dichotomy is whether each rival decides to withdraw from the fight based on a self-threshold of accumulated costs, as time, energy or injuries, or on estimates of relative chances of victory through opponent information assessment (reviewed in Arnott and Elwood 2009).

Models based on evolutionary game theory are often used to describe the decision rules (also called assessment strategies) adopted by rivals during contests (Arnott and Elwood 2009). Basically, these models are distributed in three categories: pure self-assessment, cumulative assessment, and mutual assessment models. Pure self-assessment models, as *Energetic War of Attrition* (E-WOA) (Payne and Pagel 1996, 1997) and *War of Attrition Without Assessment* (WOA-WA) (Mesterton-Gibbons et al. 1996) assume that individuals base their decision to withdraw a fight on a self-threshold of accumulated costs determined by their own condition. This threshold is directly associated to individual fighting ability and cost accrual is determined only by individual actions. Therefore, it is expected that individuals with lower fighting ability persist less and lose the fight. The *Cumulative Assessment Model* (CAM) (Payne 1998) also assumes that individuals base their decisions according to a self-threshold of cost, but according to this model, opponents' actions also play a role by inflicting costs (e.g. injury) that make this threshold to be reached faster. Consequently, the individual ability to impose and resist costs determines fighting ability. Thus, as the fighting ability of an individual increases, the higher should be the costs he inflicts on his opponent. Lastly, individuals can benefit from assessment of opponent information. Mutual assessment models, like the *Sequential Assessment Model* (SAM) (Enquist and Leimar 1983), assume that individuals assess information on their own and the opponent's fighting ability to make decisions during the fight. In this model, individuals perform less costly agonistic behaviors at the beginning of the conflict, which provide relatively unreliable information about each other, and as the conflict proceeds, they gradually escalate to more intense and costly behaviors that better indicate fighting ability. Consequently, when combatants greatly differ in fighting ability, the weaker rival may be able to perceive its weakness even with inaccurate information about the opponent and quickly withdraw from the fight. In contrast, individuals with very similar fighting ability need more reliable information to estimate which one is the weaker, and their conflict escalates to intense and costly behaviors.

A commonly adopted approach to identify which assessment strategy better explains contest dynamics is to evaluate the relationship between contest duration and fighting ability of losers and winners (Taylor and Elwood 2003). For all models, it is expected that fight duration should increase with traits linked to loser fighting ability. A significant negative relationship of winner fighting ability with contest duration excludes the hypothesis of pure self-assessment, as such strategy assumes that individuals fight according to a threshold of costs unaffected by opponents' actions. Nonetheless, this analysis does not allow differentiating between cumulative assessment and sequential assessment models, since winner fighting ability has a crucial role on both scenarios. To distinguish between these models, the better way is to analyze patterns of escalation and de-escalation within and between phases (Briffa and Elwood 2009; Fawcett and Mowles 2013), since SAM and CAM have different predictions about fight structure (Enquist and Leimar 1983; Payne 1998). SAM predicts that fights are structured in phases of increasing intensity, and that intensity remains constant within a phase, whereas fights according to CAM should escalate or de-escalate within non-injurious phases and should only escalate within phases when there is mutual injury. In addition, if fights are explained by mutual assessment (SAM), we would expect high intensity behaviors (e.g. injury imposition) to occur only in the final contest phases and when rivals have similar fighting abilities, whereas CAM does not predicts such relationship.

Many studies failed to find conclusive results about which assessment strategy better explains how rivals settle contests (Arnott and Elwood 2008 2009). In fact, some studies report mixed strategies, indicating partial support for more than one model. This may occur if individuals are able to change the assessment strategies adopted during the fight. For example, Hsu et al. (2008) demonstrated that killifish *Kriptolebias marmoratus* adopt mutual assessment at earlier stages of the fight and when fights escalates they switch to self-assessment. The analysis of overall contest alone could lead to the conclusion that contestants fight according to mutual assessment over the whole fight, as every contest present initial phases but only a subset escalate to final phases (Hsu et al. 2008). However, if self-assessment features of contest structure are present, like within-phase escalation and de-escalation in the final phase, it may raise a scenario where contest structure provides evidences for one model and contest duration support another model.

In the present work, we used males of the cricket *Melanotes ornata* Desutter-Grandcollas 1993 (Grylloidea: Phalangopsidae) as model organisms. Males of this species occupy mating territories while performing acoustic signals to attract mates, and actively defend these territories against invading males (G Lobregat, personal observation). Here we tested predictions of pure self-assessment, cumulative assessment and sequential assessment models, to explain the assessment strategies adopted by rivals during agonistic behaviors of territory defense. For this, we first identified individual attributes that better determined the probability of victory in order to identify the best proxy of fighting ability. Then, we evaluated fight structure (*i.e.* number of distinct behavioural phases and pattern of escalation and de-escalation between such phases) and the relationship between fighting ability of individuals and contest duration (for both the whole fight and for each fighting phase).

METHODS

Study site and species

We conducted field observations at the Estação Biológica de Santa Lúcia (19º57'S, 40º32'W) and Reserva Biológica Augusto Ruschi (19º54'26"S, 40º33'11"W), Santa Teresa municipality, Espírito Santo state, South-eastern Brazil. These locations are Atlantic Forest nature reserves with areas of 440 and 3600 ha (IBAMA 1995; Mendes and Padovan 2000), respectively. Both reserves are classified as dense ombrophilous mountain forests (Velozo and Góes-Filho 1982). We conducted the study between September and November 2015, period of the year that represents the apex of reproduction phase of *M. ornata* (TG Kloss, personal observation).

Melanotes ornata is a nocturnal cricket species, whose known distribution is restricted to these conservation units (Desutter-Grandcolas, 1993). Individuals of this species occur in a wide range of habitats, but they are most commonly found on natural shelters present in tree trunks, like cavities in the suber or beneath leafs surrounding these trunks. When producing acoustic signals to attract mates, males often occupy more exposed sites in the trunks. When they feel threatened, they go back to their shelters (G

Lobregat, personal observation). Singing male crickets actively defend their calling sites against the approximation of conspecific males (see Results section for a detailed description of the behaviors adopted during agonistic interactions).

Field experiment

To evaluate conflicts in natural conditions, we performed an experiment to induce contests in the field. For this, we captured males that were emitting calling songs, irrespective of their location, and held each male captive for a maximum period of 10 min. During this period, we searched for another calling male and captured it to pair with the first captured male. To facilitate identification of individuals during fight observation, we marked one individual with colored glue on its pronotum, using a wood stick. Afterwards, to avoid differential handling effects on individuals' behavior, we removed the glue from the stick and touched it on the pronotum of the other individual. After these procedures, we waited 5 min and then positioned the first captured male in the calling site of the second one. We waited five additional minutes and released the second male in the same place to observe their behavior. We opted for arranging fighting pairs ourselves, instead of observing natural fights, because natural occurrences of fights were difficult to observe, especially if we wanted to observe fights from the beginning. We observed fighting behavior at a minimum distance of 0.5 m with red flash light to minimize the influence of our presence. We took verbal notes with a voice recorder whenever an individual performed an activity (different activities are described in the results section) and used these audio files to characterize different contest phases (based on the behaviors adopted by males), to estimate contest duration and duration of each phase. We considered the beginning of a contest as the touch of antennae between individuals and the end when one of the individuals quickly stepped away more than 10 cm from its rival. Because some individuals could step away and return to continue the fight, we stipulated a minimum period of 1 min observing the two contestants staying apart to consider that the fight had ended. We based this period on previous field observations where the withdrawal for more than 1 min always led to resuming of the contest. We excluded from our data the conflicts in which it was not possible to discriminate which individual won the fight and conflicts in which individuals stayed 10 cm apart for more than 1 min. This manner, we obtained 14 field observations of male contests. After observations, we captured the individuals, took them to the lab and euthanized in the freezer, at -5 ºC.

Laboratory experiment

To complement the information obtained in the field, we induced fights between males in the lab. For this, we transferred field captured individuals to the lab and individualized them in plastic recipients (10 cm diameter X 12 cm height). The laboratory was localized inside the Estação Biológica de Santa Lúcia, one of the conservation units where individuals were caught. Temperature and humidity followed the local environmental fluctuation during the sampling period (12.9 to 27.1 ºC; 42 to 100% air relative humidity; INMET 2018). We separated recipients with a minimum distance of 1 m, and provided humidified cotton, shelter and food *ad libtum*. Individuals were held captive for at least 24 h before conflicts. We captured 58 individuals to form 29 randomly selected pairs, of which 25 resulted in conflicts. We also captured females in the field to use them to motivate males to fight. Such procedure was taken due to previous observations that the chances of both males starting to fight in lab conditions were low in the absence of female stimulus. Females were held captive in separate recipients for at least 24 h before using them in the experiment. Before pairing males, we marked each of them with a drop of colored glue on their pronotum, each one with a different color (red or yellow). Ten minutes after marking, we separately presented the same female to each of these males, by placing her in each male's recipient, one after the other. When males started courtship behaviors (brief and slow movements towards the female and emission of low intensity acoustic signals), we waited for 2 min and removed the female from the recipient to avoid pre-fighting copula. When males did not present courtship behavior after 10 min of female exposition, we also removed the female. These males (n=16) were also used in the conflicts and did not present differences in fighting behavior compared to males that had presented courtship behavior. We used six females in this procedure and we never presented the same female to two consecutive pairings. We staged contests in a plastic bucket (15 cm diameter X 15 cm height) surrounded by white paper with sand and dry leaves as substrate. We carefully placed individuals at the same time from the top opening of the arena and observed their behavior after antennal touch. We carried out all contests in the afternoon (between 2 and 6 p.m) and filmed them with a Nikon D3100 camera. Contest description and criteria for recognition of conflicts' beginning and ending were the same of field observations. After observations, individuals were euthanized in the freezer, at -5 ºC.

Mensuration of morphological and physiological attributes

To evaluate possible determinants of male fighting ability, we measured hind femur length, fat content and muscle content of each individual (n=78). We evaluated hind femur length because previous observations showed that the hind legs have an important role during the fight (see Results below). We evaluated fat content and muscle content because the first might be related to energetic reserves spent along the conflicts and the second might be associated to the ability to impose higher damage during the fight. Immediately after euthanizing individuals, we measured hind femur length using a digital caliper (precision of 0.01 mm). To estimate fat content, we dried the individuals in an oven at 60 ºC for 48 h and then weighed their thorax, abdomen and hind legs in an analytic balance (precision of 0.0001 g). After this, we put these samples in 10 ml of chloroform to extract lipids, dried them again at 60 ºC for 48 h and re-weighed them. To estimate muscle content, the same samples with lipids already extracted were weighed before and after immersion in a solution of 0.3 M sodium hydroxide. Fat and muscle extraction followed the method proposed by Peixoto and Benson (2012). We considered fat content as the weight difference before and after chloroform extraction and muscle content as the weight difference before and after immersion in sodium hydroxide. To obtain values of fat content and muscle content regardless of individual mass, we performed linear regressions between these attributes and dry weight, and used the residuals estimated from these linear regressions, instead of using the absolute values of each individual attribute (Marden and Chai 1991; Marden and Rollins 1994).

Statistical analysis

Traits associated to fighting ability

To determine which trait better indicates fighting ability for both field (n=14) and laboratory (n=25) observations, we adjusted binomial generalized linear mixed effects models (GLMMs) with probability of victory as binary response variable (0 = defeat, 1 = victory). We included the environment where each contest took place (field or laboratory) as random factor and adjusted alternative models that differed in the following fixed effects: hind femur length, residual fat content and residual muscle content. Since each contest comprehends a pair of opponents, we performed a data handling procedure as proposed by Kemp (2000) to restrict the number of replicates to the number of pairings, instead of analyzing all individuals regardless of each pair they pertain. For each contest, we randomly assigned the focal male as the winner or the loser of the contest, so that each contest rendered a single contest result, either victory, when the focal contestant was a winner, or defeat, when the focal contestant was the loser. The total set of contests was, therefore, divided in two sets with the same number of observations, regarding which result (loser or winner) the focal males achieved. We assigned a value of 1 to focal winners' contests and 0 to focal losers' contests, for the variable on contest result. We calculated the difference between focal males and their non-focal rivals for each explanatory fixed variable. In this way, if some attribute increased winning chances, contests in which the focal male was the winner should present positive values for the difference in such attribute, while contests in which the loser was the focal male should present negative values. We used Akaike Information Criterion corrected for small samples (AICc) to select the candidate model that better described the relationship between the difference in an attribute and the probability of winning, and used the selected attribute for further analyses of game theory models' predictions. We also evaluated significance of the selected attribute with Likelihood Ratio Test (LRT) in order to verify if the probability of victory was indeed associated to such attribute. All data were subjected to preliminary exploratory analyses, as recommended by Zuur et al. (2009).

Distinction among game theory models

To test model predictions related to contest duration, we performed, for all contest observations (n=39), a multiple regression of fight duration against measures of the attribute identified in the previous analysis as determinant of fighting ability for both winners and losers (Taylor and Elwood 2003). Self-assessment predicts that contest duration should increase with loser fighting ability and be unrelated to winner fighting ability. In counterpart, cumulative and mutual assessment predict that contest duration should increase with loser fighting ability and decrease with winner fighting ability. Mutual assessment also predicts that the effects of loser and winner fighting abilities on contest duration should present opposite signals but similar magnitudes, so we estimated the slope for each variable. We performed these analyses both for overall contest duration and for duration of each behavioral phase. For separated phase analyses, we also included interaction terms between fighting ability measures of winners and losers and a categorical variable informing if the contest advanced to the next phase or not. Including this interaction in the model allowed us to verify if the relationship between phase duration and fighting ability differed between contests that resumed in the evaluated phase and contests that advanced to the subsequent phase.

We also tested the sequential assessment prediction that the advance to subsequent contest phases and the use of high-cost behaviors have higher probability to occur when contestants have very similar fighting abilities. For this, we adjusted a logistic regression between the probability of fights advancing to subsequent phases (1 = yes, 0 = no) and the difference in fighting ability between opponents. We did the same for the probability of kick exchange between opponents, as this behavior appeared to be the most costly in their contests (see Results). For these two analyses, we expected a negative relationship between contest phase advance and traits related to fighting ability if fights follow mutual assessment, and no relationship if they follow cumulative assessment (Enquist and Leimar 1983). Models with contest or phase duration as response variable did not present adequacy of residuals regarding normality and homoscedasticity, which we corrected by log-transforming contest duration. For every statistical model in these analyses, we adjusted environment as random factor with two levels (lab x field). We performed all statistical analyses within R (R Core Team 2017).

Ethical note

Experimental manipulation of crickets and fieldwork were performed with permission from the System of Authorization and Information on Biodiversity (SISBIO/ICMBio Authorization No. 49634-3, Brazil) and complied with the current legal and ethical requirements for animal welfare in Brazil. Experiments were conducted at the site of cricket occurrence in the field or in the laboratory located within the Estação Biológica de Santa Lúcia/MBML, so as to minimize stress due to transportation or due to alteration of climatic conditions, preventing injuries and maintaining animal welfare by avoiding unusual changes in temperature and humidity. Captured individuals that were eventually not used in laboratory procedures or that did not fight in an observation attempt were released in their habitat. The studied species, *M. ornata,* is not endangered or protected in Brazil.

RESULTS

Fighting behavior

Males of *Melanotes ornata* presented fighting behavior comprising two phases, which always followed the same sequence of execution. In the first phase (n=39), after brief mutual antennal touch, individuals changed their orientation towards the opponent, subsequently elevated their body ventro-dorsally from the ground and presented slow-movement lifting of their hind legs. While doing so, rivals moved around each other gradually positioning their back towards each other while pointing the hind legs towards the opponent. In this phase, there were also acceleration of antennal movements and repeated touching on the opponent's antennae and other body parts with its own antennae. In the second phase (n=20, Fig. 1), individuals got closer, and crossed their hind legs, starting a series of pushes and kick attempts, with intermittent emission of acoustic signals. In three contests there were also frontal assaults, wrestling and biting.

Figure 1 – Males of *Melanotes ornata* in an escalated contest. Individuals position their posterior region towards each other, cross their hind legs and start a series of pushes and kicks with hind legs. Individuals also raise their tegmen and emit intermittent acoustic signals of variable duration (represented by the individual in the right).

Whenever an opponent continued to the second phase, the rival also changed its behavior or fled from the fight. Once individuals entered the second phase, they never returned to the previous one (*i.e.* there was no de-escalation between phases). At all conflicts, when one individual successfully kicked his opponent away, or by any reason, individuals distanced from each other, one or both individuals started to emit acoustic signals until they got close again. After one of the males withdrew from the fight, it fled whenever it met its opponent again. The sequence of behaviors executed was the same in field and laboratory observations. The environment where fights took place (field or lab) had no effect on the determinants of winning probability (variance associated to environment as random factor < 0.0001) or on contest duration (variance associated to environment as random factor < 0.0001).

Attributes associated to fighting ability

Winners presented higher mean values than losers for all three attributes (Table 1). Among candidate models, the most parsimonious was the one with hind femur length difference (Table 2), distinguishing itself from the other candidate models by more than

16 Δ AICc. Femur length increased the probability of winning (LRT= 17.001; $p < 0.001$; Fig. 2). Thus, we used this variable in the following analyses of game theory models' predictions. We observed no effect of territory ownership between owners and intruders on the probability of winning in field observations (owner as winner: 7 conflicts; intruder as winner: 7 conflicts).

Table 1. Attribute values of winner and loser males of *Melanotes ornata* in field and laboratory contest observations (n=39).

	Values	**Hind femur length (mm)**	**Fat content (mg)**	**Muscle content (mg)**
Winners				
	Mean	11.07	9.61	27.71
	SD	0.57	1.86	5.97
Losers				
	Mean	10.64	8.77	24.65
	SD	0.62	1.7	4.91

Table 2. Results of AICc for the relationship between probability of victory of focal male and difference between focal and non-focal males for each evaluated predictor in *Melanotes ornata* contests.

Predictor	**AICc**	**Δ_i**	**w_i**
Femur size	43.72	0	1
Muscle content	59.84	16.13	0
Fat content	59.92	16.2	0

AICc: Akaike's Information Criterion value corrected for small sample sizes; Δ_i: AICc difference between the most parsimonious model and model *i*; w_i: Akaike weight of model *i*.

Contest duration and structure

Contest duration was positively associated to hind femur length of losers ($\chi^2 = 12.92$; $p = 0.001$; $b = 0.85$ $SE = 0.22$; Fig. 3a) and negatively associated to hind femur length of winners ($\chi^2 = 6.19$; $p = 0.013$; $b = -0.66$ SE = 0.26; Fig. 3b). Out of 39 observed contests, 20 escalated to the second phase. Among the 20 fights that escalated to the second phase, six ended before there was kick exchange, as losers retreated briefly after contest escalation. The probability of fights escalating to the second phase was negatively associated to the difference in hind femur length between opponents ($\chi^2 = 6.19$; $b = -1.52$ SE = 0.68; $p = 0.013$; Fig. 4a). The same was true for the probability of kick exchange and difference in hind femur length between opponents ($\chi^2 = 8.08$; $b = -2.14$ SE = 0.94; $p = 0.004$; Fig. 4b).

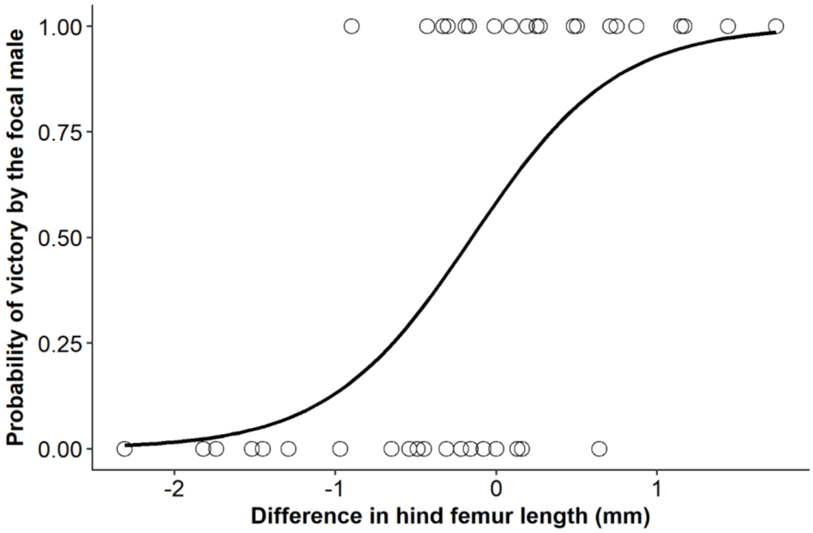

Figure 2 – Relation between focal male probability of victory and difference in residual hind femur length between focal and non-focal males of *Melanotes ornata* contests. Positive values in X-axis represent contests where the focal male had proportionally larger hind femur size than its rival, and negative values represent the opposite.

Considering only the first phase, duration was positively associated to hind femur length of losers ($\chi^2 = 6.9$; $p = 0.008$; $b = 0.62$ SE = 0.25; Fig. 5a), and there was no difference in this relationship between contests that advanced to the second phase and contests that ended in the first phase ($\chi^2 = 1.43$; $p = 0.23$). Duration of first phase was negatively associated to hind femur length of winners in contests that did not progress to the next phase (χ² = 6.34; b = -0.9 SE = 0.35; p = 0.011; Fig. 5b), whilst this relation was not significant when contests advanced to the next phase (χ² = 1.17; b = 0.27 SE = 0.28; p = 0.29; Fig. 5b). Duration of the second phase was not associated with hind femur length of losers (χ² = 3.25; p = 0.071; b = 0.58 SE = 0.33; Fig. 5c) nor winners (χ² = 2.49; p = 0.114; b = -0.44 SE = 0.29; Fig. 5d).

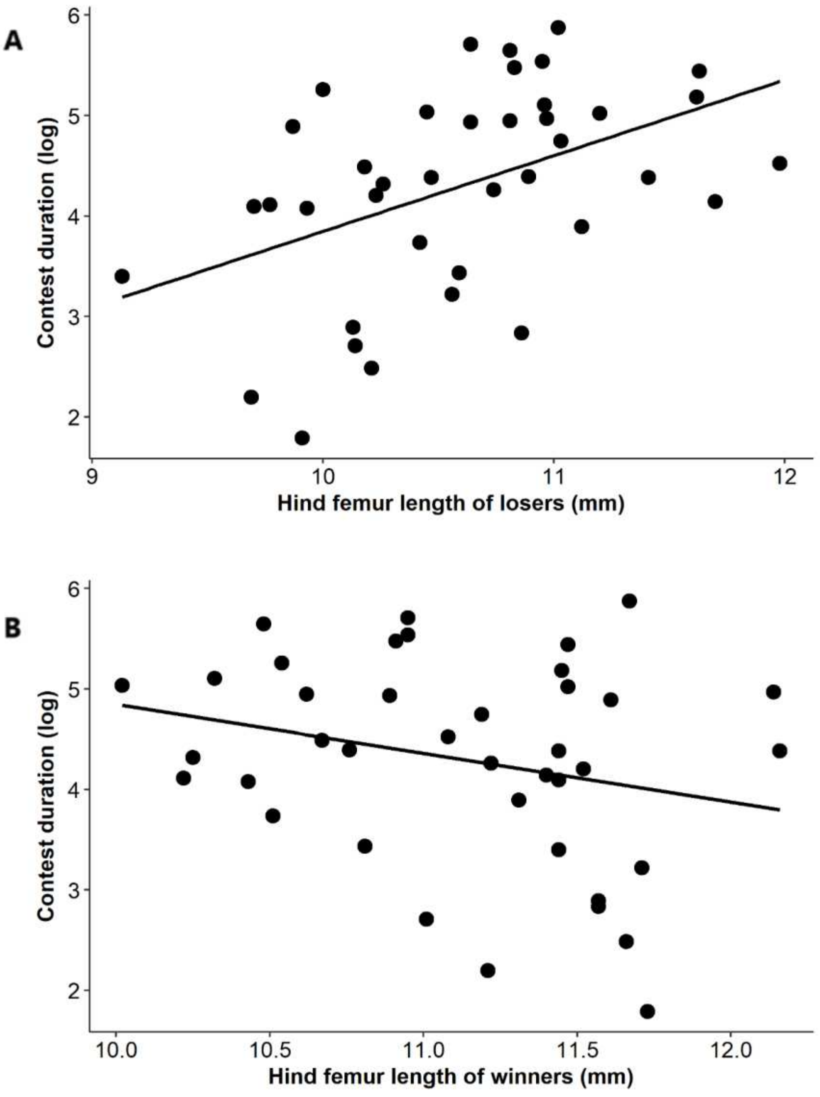

Figure 3 – Relation between overall contest duration (log) and **(A)** hind femur length of losers and **(B)** hind femur length of winners in *Melanotes ornata* contests.

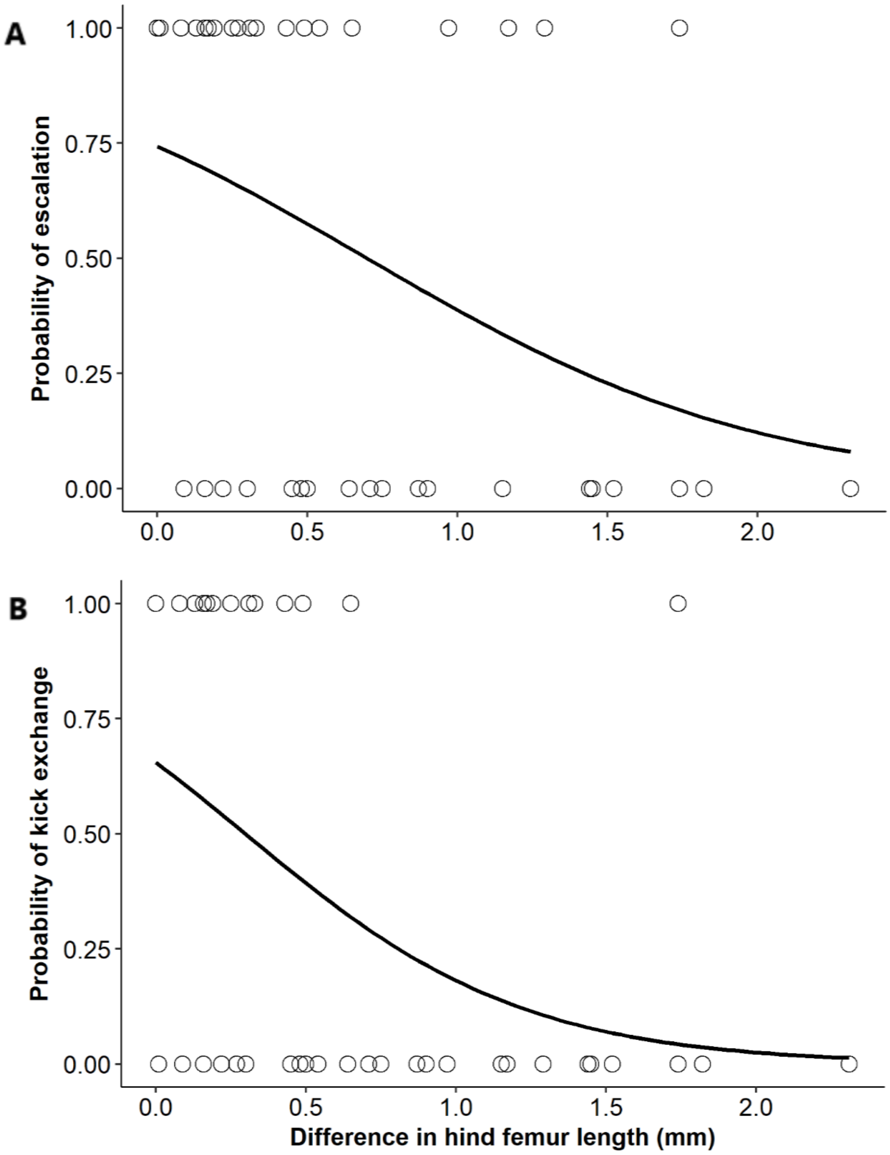

Figure 4 – Contest structure of *Melanotes ornata* contests. Relation between difference in hind femur length and (**A**) the probability of contest escalation to the final phase and (**B**) the probability of kick exchange between contestants.

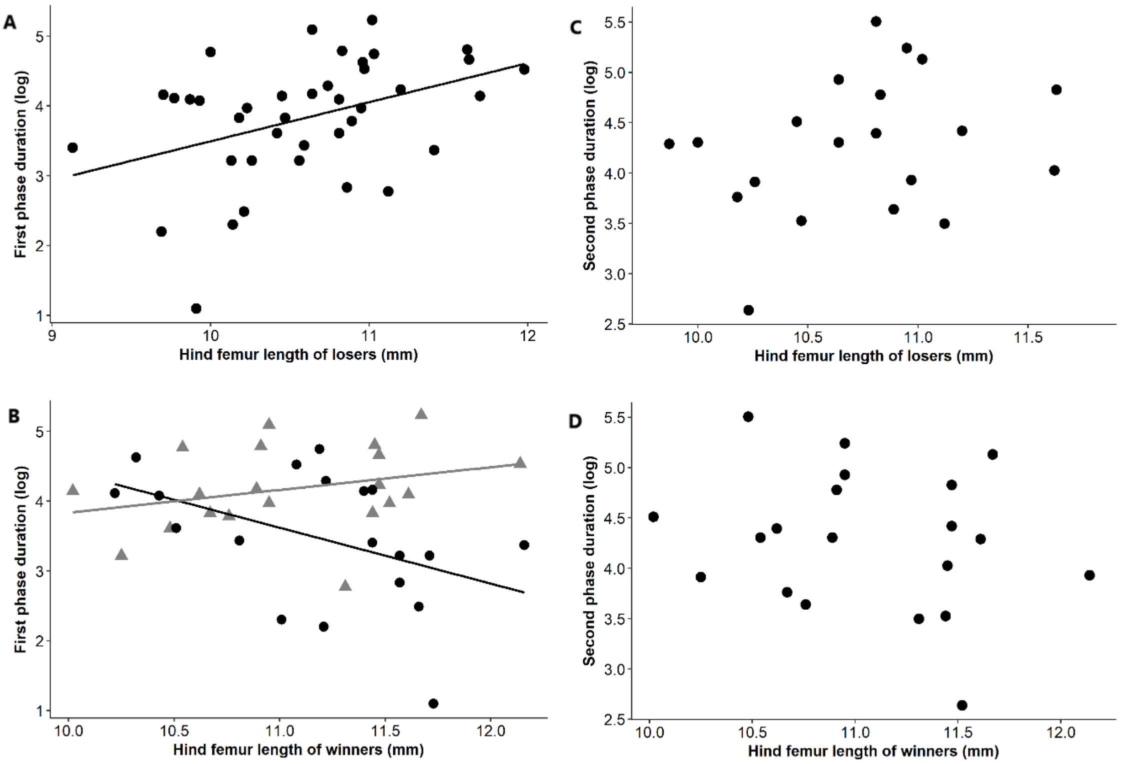

Figure 5 – Relationship between duration of phases and hind femur length of *Melanotes ornata* males. First phase duration relation with (**A**) hind femur length of losers and (**B**) hind femur length of winners. Second phase duration relation with (**C**) hind femur length of losers and (**D**) hind femur length of winners. In (B), grey triangles represent contests that escalated to the second phase, whereas black circles represent contests that finished in the first phase. Regression lines were plotted only for significant relationships.

DISCUSSION

We demonstrated that individuals of *Melanotes ornata* with lengthier hind femurs present higher chances of winning, indicating that such trait is an important determinant of male fighting ability. When evaluating the relationship between overall contest duration and hind femur length, our results indicate that *M. ornata* contests behave according to the sequential assessment model. We found opposite effects of loser and winner hind femur length on fight duration, and a negative relationship between probability of kick exchange and difference in hind femur length between contestants, with no de-escalation between phases. These results indicate that as contestants' difference in fighting ability decrease, fights take longer to resolve and involve the performance of high-cost strategies (i.e. kick exchange). However, when evaluating contest phases separately, the relationship between fight duration and hind femur length remained only in the first phase of the fight (particularly for contests that did not escalate), but no relationship was found in the second phase. We propose that individuals mutually access each other's fighting ability in the first phase of the fight, and when their fighting ability is similar, contests escalate to the second phase, in which individuals stop performing mutual assessment.

The prominent use of hind legs in the first phase, and the use of these appendices for kicking in escalated contests, show its important role in *M. ornata* contests and appear to be the main attribute accessed in the initial phase. Hind legs could be associated to the ability of imposing injury against the opponent (Irschick et al. 2007; Lailvaux and Irschick 2006), or the ability of successfully kicking the rival out of the trunk to the ground. Thus, assessment of this attribute probably provides adequate information about the potential costs of persisting in the contest. In counterpart, fat and muscular content were poorer predictors of winning probability in *M. ornata* contests. Fat reserves are usually related to the ability to persist for longer periods in endurance fights, particularly for flying insects that present aerial contests (e.g. Marden and Waage 1990; Peixoto and Benson 2011). Since males of *M. ornata* perform activities of apparently low energetic demand in the first phase and damage seems to be of higher importance in the second phase, fat is probably of little importance to fighting ability. The lack of relationship between residual muscle content and fighting ability indicate that the amount of muscular tissue alone does not reflect the ability of imposing injury with kicks. In this sense, it seems that it is not muscle investment per se that affects winning chances, but the absolute investment in femur length, probably reflecting the mechanic lever effect of femur length on kicking strength

Although the entire contest between *M. ornata* males is apparently well explained by sequential assessment rules, our evaluation per phase indicates that rivals change assessment strategy when fights escalate. In the first phase, the opposite effects of loser and winner hind femur length on contest duration, as well as the increased probability of fights escalating to a high intensity final phase when contestants had similar fighting abilities, provide strong support for mutual assessment before fight escalation. However, when fights escalated, no relationship between duration and hind femur length was found, evidencing a change in assessment rules. Because every contest presented the first phase, it may be that the conclusion regarding overall contest duration was biased by the pattern presented in the first phase.

Switching assessment strategies was first suggested by Morrel et al. (2005) studying contests of the fiddler crab *Uca mjoebergi*. Although these animals seem to follow prediction from CAM, fights were size-assortative, suggesting that individuals access opponent information before entering escalated contests. Other studies evaluating contest phases separately also observed switching strategies along the contest, but no pattern of whether initial phases are better represented by mutual or self- assessment was found (Hsu et al. 2008; McGinley et al. 2015; Yasuda et al. 2012). In *M. ornata*, the first phase fits sequential assessment predictions, whereas the second phase apparently does not follow predictions of any model. Because the second phase occurs between rivals with similar hind femur lengths, there might be additional asymmetries, not related to fighting ability, that affect wining chances. In particular, motivational asymmetries may affect how much each individual persists in the second phase (reviewed in Arnott and Elwood 2008). In this manner, males of *M. ornata* would be primarily accessing fighting ability in the first phase and, when both rivals decide to escalate, more motivated individuals might win the fight. We should, however, not exclude the possibility that the escalated phase follows pure self-assessment rules. Later contest phases present smaller sample sizes, since only a subset of the observed contests escalated. Considering the marginal p-value (p=0.07) of loser hind femur length effect on second phase duration, larger sample size might reveal evidence for pure self-assessment predictions (Briffa and Elwood 2009; Taylor and Elwood 2003) . If such scenario is true, *M. ornata* males would mutually access each other in the first phase, before advancing to escalated phases, where they would fight until their self-threshold of costs was achieved. This should make sense when we consider that as the difference in fighting ability decreases, it is more difficult for individuals to detect this asymmetry (Enquist and Leimar 1983), and other assessment strategies should provide more confident information.

Some authors criticize the possibility of mutual assessment evolving in invertebrate species (Elwood and Arnott 2012), but others suggest it as a cognitively simple process of continuous estimation of relative fighting ability (Fawcett and Mowles 2013). According to the latter, mutual assessment is easily reachable when the physiological state of individuals is affected by the combined effect of information assessed from opponent displays and information about its own physiological status, like depletion of available energy (Briffa 2008) or of neurotransmitters associated to aggressiveness (Rillich and Stevenson 2015). In the first phase of *M. ornata* contests, males present frequent touch of their antennae on rival's body and hind legs. In other crickets, although antennal touch triggers aggressive behaviors (Adamo and Hoy 1995) and allows access of adversary's motivation to enter the fight (Hofmann and Schildberger 2001; Tachon et al. 1999), there are no studies demonstrating assessment of fighting ability through antennal touch. Notwithstanding, cricket females are able to predict dominance hierarchy between males before their fight through chemical cues (Kortet and Hedrick 2005), which are mainly accessed with antennal chemoreceptors (Balakrishnan and Pollack 1997). Therefore, male *M. ornata* may use a similar chemo-sensorial mechanism to estimate fighting ability of their opponent. Information exchange could also occur by the lifting of hind legs in the initial phase, exhibiting it to the rival and facilitating the assessment of their size via antennal touch.

In conclusion, our results highlight the importance of evaluating the relationship between costs and the ability to pay these costs for separated contest phases. This is supported by some studies that evaluated overall contest duration, but found evidences for more than one model along the contests (Briffa 2008; Palaoro et al. 2014; Pratt et al. 2003; Stuart-Fox 2006). Since fights of the majority of species present more than one behavioral phase (Hardy and Briffa 2013), we argue that most studies which tried to evaluate a single assessment strategy over the whole fight, might be masking relationships between fighting ability and duration in separate phases. Therefore, switching of assessment strategies may occur in a broader range of species.

REFERENCES

Adamo SA, Hoy RR. 1995. Agonistic behaviour in male and female field crickets, *Gryllus bimaculatus*, and how behavioural context influences its expression. Anim Behav. 49:1491–1501.

Arnott G, Elwood RW. 2008. Information gathering and decision making about resource value in animal contests. Anim Behav. 76:529–542.

Arnott G, Elwood RW. 2009. Assessment of fighting ability in animal contests. Anim Behav. 77:991–1004.

Balakrishnan R, Pollack GS. 1997. The role of antennal sensory cues in female responses to courting males in the cricket *Teleogryllus oceanicus*. J Exp Biol. 200:511–522.

Briffa M. 2008. Decisions during fights in the house cricket, Acheta domesticus: mutual or self assessment of energy, weapons and size? Anim Behav. 75:1053–1062.

Briffa, M, Elwood RW. 2009. Difficulties remain in distinguishing between mutual and self-assessment in animal contests. Anim Behav. 77:759–762.

Desutter-Grandcolas L. 1993. *Melanotes* et *Koilenoma*, deux nouveaux genres de Luzarinae à écologie nouvelle pour la sous-famille (Orthoptera, Grylloidea, Phalangopsidae). Bull Soc entomol Fr. 98:275–286.

Elwood RW, Arnott G. 2012. Understanding how animals fight with Lloyd Morgan's cannon. Anim Behav. 84:1095–1102.

Enquist M, Leimar O. 1983. Evolution of fighting behaviour: Decision rules and assessment of relative strength. J Theor Biol. 102:387–410.

Fawcett TW, Mowles SL. 2013. Assessments of fighting ability need not be cognitively complex. Anim Behav. 86:e1–e7.

Hardy ICW, Briffa M. 2013. Animal contests. New York: Cambridge University Press.

Hofmann HA, Schildberger K. 2001. Assessment of strength and willingness to fight during aggressive encounters in crickets. Anim Behav. 62:337–348.

Hsu Y, Lee SP, Chen MH, Yang SY, Cheng KC. 2008. Switching assessment strategy during a contest: fighting in killifish *Kryptolebias marmoratus*. Anim Behav. 75:1641–1649.

IBAMA 1995. Plano de Manejo da Reserva Biológica Augusto Ruschi. Retrieved from http://www.icmbio.gov.br/portal/images/stories/imgs-unidades-coservacao/rebio_augusto-ruschi.pdf

INMET 2018. Consulta Dados da Estação Automática: Santa Teresa (ES). Retrieved from http://www.inmet.gov.br/sonabra/pg_dspDadosCodigo_sim.php?QTYxMw==

Irschick DJ, Herrel A, Vanhooydonck B, Damme RV. 2007. A functional approach to sexual selection. Func Ecol. 21:621–626.

Kemp DJ. 2000. Contest behavior in territorial male butterflies: does size matter? Behav Ecol. 11:591–596.

Kortet R, Hedrick A. 2005. The scent of dominance: Female field crickets use odour to predict the outcome of male competition. Behav Ecol Sociobiol. 59:77–83.

Lailvaux, SP, Irschick DJ. 2006. A functional perspective on sexual selection: insights and future prospects. Anim Behav. 72:263–273.

Marden JH, Chai P. 1991. Aerial predation and butterfly design: how palatability, mimicry, and the need for evasive flight constrain mass allocation. Am Nat. 138:15–36.

Marden JH, Rollins RA. 1994. Assessment of energy reserves by damselflies engaged in aerial contests for mating territories. Anim Behav. 48:1023–1030.

Marden JH, Waage JK. 1990. Escalated damselfly territorial contests are energetic wars of attrition. Anim Behav. 39:954–959.

Maynard-Smith J. 1982. Evolution and the Theory of Games. Cambridge: Cambridge University Press.

Maynard-Smith J, Parker GA. 1976. The logic of asymmetric animal contests. Anim Behav. 24:159–175.

McGinley RH, Prenter J, Taylor PW. 2015. Assessment strategies and decision making in male-male contests of *Servaea incana* jumping spiders. Anim Behav. 101:89–95.

Mendes SL, Padovan MDP. (2000). A Estação Biológica de Santa Lúcia, Espírito Santo. Bol Mus Biol Mello Leitão. 11:7–34.

Mesterton-Gibbons M, Marden JH, Dugatkin LA. 1996. On wars of attrition without assessment. J Theor Biol. 181: 65–83.

Morrell LJ, Backwell PRY, Metcalfe NB. 2005. Fighting in fiddler crabs *Uca mjoebergi*: What determines duration? Anim Behav. 70:653–662.

Palaoro AV, Dalosto MM, Costa JR, Santos S. 2014. Freshwater decapod (*Aegla longirostri*) uses a mixed assessment strategy to resolve contests. Anim Behav. 95:71–79.

Parker GA. 1974. Assessment strategy and the evolution of animal conflicts. J Theor Biol. 47:223–243.

Payne RJH. 1998. Gradually escalating fights and displays: the cumulative assessment model. Anim Behav. 56:651–662.

Payne RJH, Pagel M. 1996. When is false modesty a false economy? An optimality model of escalating signals. Proc Royal Soc Lond B. 263:1545–1550.

Payne RJH, Pagel M. 1997. Why do animals repeat displays? Anim Behav. 54:109–119.

Peixoto PEC, Benson WW. 2011. Fat and body mass predict residency status in two tropical Satyrine butterflies. Ethology. 117:722-730.

Peixoto PEC, Benson WW. 2012. Influence of previous residency and body mass in the territorial contests of the butterfly *Hermeuptychia fallax* (Lepidoptera: Satyrinae). J Ethol. 30:61–68.

Pratt AE, McLain DK, Lathrop GR. 2003. The assessment game in sand fiddler crab contests for breeding burrows. Anim Behav. 65:945–955.

R Core Team. (2017). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Rillich J, Stevenson PA. 2015. Releasing stimuli and aggression in crickets: octopamine promotes escalation and maintenance but not initiation. Front Behav Neurosc. 9:95.

Stuart-Fox D. 2006. Testing game theory models: fighting ability and decision rules in chameleon contests. Proc Royal Soc Lond B. 273:1555–1561.

Tachon G, Murray A, Gray DA, Cade WH. 1999. Agonistic displays and the benefits of fighting in the field cricket, *Gryllus bimaculatus*. J Insect Behav. 12:533–543.

Taylor PW, Elwood RW. 2003. The mismeasure of animal contests. Anim Behav. 65:1195–1202.

Velozo HP, Góes-Filho L. 1982. Fitogeografia brasileira: classificação fisionômica-ecológica da vegetação neotropical. Salvador: Projeto Radambrasil.

Vieira MC, Peixoto PEC. 2013. Winners and losers: A meta-analysis of functional determinants of fighting ability in arthropod contests. Func Ecol. 27:305–313.

Yasuda C, Takeshita F, Wada S. 2012. Assessment strategy in male-male contests of the hermit crab *Pagurus middendorffii*. Anim Behav. 84:385–390.

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. 2009. Mixed effects models and extensions in ecology with R. New York: Springer-Verlag.

CHAPTER THREE

Aggressive song signals motivation in contests of a Neotropical cricket

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ABSTRACT

Animal contests usually present the emission of displays that provide information about the individuals and may facilitate assessment of rivals attributes. As signal production incur costs to the signaler, individuals able to pay higher costs may produce higher intensity or longer lasting signals. Thus, aggressive signals are expected to provide cues about attributes associated to the ability or the willingness of paying such costs, such as fighting ability and motivational state. In the present work, we investigate the information content of aggressive sound displays of *Melanotes ornata* male crickets. We measured sound frequency and pulse rate (i.e. number of sound-producing wing closures in one second) of the aggressive song, as well as total signaling effort, and evaluated their relationship with body size (a proxy of fighting ability) and motivation. Sound parameters were not associated to body size, indicating that these signals do not inform individual fighting ability. In contrast, motivated individuals emitted sound signals with higher signaling effort and chirp rates. This result demonstrates that aggressive sound may provide information about motivational status in this species. As the emission of aggressive sound signals is restricted to escalated fights in *M. ornata*, which most frequently occur when contestants have small differences in fighting ability, differences in motivational state probably have a higher influence in the outcome of escalated fights. Thus, informing motivation to the opponent may accelerate the loser's decision to retreat in a greater extent than signaling fighting ability.

KEYWORDS: displays, animal fight, Orthoptera, stridulation, signaling effort

INTRODUCTION

Displays are commonly performed by animals with the primary function of transmitting information to a target recipient and influence its decision (Johnstone, 1995; Smith & Harper, 1995; Vehrencamp, 2000; Zahavi, 1975). In aggressive contexts, such displays are expected to inform some aspect of the signaler's quality, which is used by the receiver in its decision to stay in the fight or flee from the rival (Enquist & Leimar, 1987; Hurd, 1997). Game theory models suggest that the chances of an individual winning a fight is mainly determined by two factors: (i) fighting ability, which is the combination of attributes that permit a higher payment of fight-associated costs or a greater imposition of costs to the rival , and (ii) motivational state, which is influenced by how an individual values the contested resource and affects the amount of costs he is willing to pay for its possession (Enquist & Leimar, 1983, 1987; Leimar & Enquist, 1984; Maynard-Smith & Parker, 1976; Mesterton-Gibbons et al., 1996; Payne & Pagel, 1996).

According to the handicap principle (Zahavi, 1975; Zahavi & Zahavi, 1999), the honesty of displays is maintained by costs or constraints that minimize the chances of weak individuals perform high-quality displays, in a way that the parameters of such signals should be linked to the quality of individuals. Acoustic signaling is one of the most conspicuous form of displays, performed by a vast number of taxonomic groups (Searcy & Andersson, 1986). Several studies demonstrate a relationship between the form and intensity of acoustic displays and signalers' fighting ability or motivation (Brown et al., 2006; Clutton-Brock & Albon, 1979; Reichert, 2013, 2014; Vehrencamp, 2000). For example, peak sound frequency usually has a negative association to the size of sound-producing structures in crickets and frogs (Koch et al., 1988; Ryan, 1988). As body size is correlated to the size of these structures, individuals are incapable of modulating their sound frequency largely, so that such parameter will inevitably inform fighting ability

(Moradian & Walker, 2008; Reichert, 2014). Other sound characteristics like signaling effort, sound amplitude and temporal properties of sound are determined by energetic constraints in a way that signalers in a better condition or in a higher motivational state perform longer, louder or faster acoustic signals (Clutton-Brock & Albon, 1979; Eberhardt, 1994; Mowles, 2014; Wyman et al., 2008) .

In many cricket species, males produce pure-tone acoustic signals by rubbing specialized structures of their fore wings that promotes resonance of wings' specialized areas (Koch et al., 1988). The acoustic repertoire of these insects include distinct songs associated to different contexts of which the most common are the calling song and the courtship song associated to mating, and the aggressive song which is performed along aggressive contests (R. D. Alexander, 1961; Richard D. Alexander, 1962). Several studies found a relationship between sound parameters of cricket song and attributes associated to individual quality, suggesting that such signals may contain information about sender attributes. In many species of *Gryllus*, nutritional restriction affects the temporal pattern of sound emission and total calling effort of individuals (Hedrick, 2005; Holzer et al., 2003; Judge et al., 2008; Scheuber et al., 2003a; Wagner & Hoback, 1999), as well as other energetic constraints like parasitoid and bacterial infection (Cade, 1984; Jacot et al., 2004; Orozco & Bertram, 2004). When such constrain is restricted to juveniles, individuals develop smaller harps (the resonance driver of crickets' sound production) in their wings, leading to a higher carrier frequency in *Gryllus campestris* (Scheuber et al., 2003b). Temporal and spectral parameters of cricket songs are also related to body size and body condition in some species (Bertram & Rook, 2012; Judge, 2011; Simmons & Zuk, 1992), and females are able to discriminate these parameters in their mating decision (Gray, 1997; Leonard & Hedrick, 2010; Rantala & Kortet, 2003; Tregenza et al., 2006). However, the vast majority of studies evaluated the information content of the calling song, and little is known about what information acoustic signals performed in aggressive interactions between males may contain (Brown et al., 2006). Furthermore, most studies focused on only three genus of field crickets (*Acheta, Gryllus* and *Teleogryllus*), which are ecologically very similar, hindering the understanding of how different life histories and sexual behaviors is associated to the acoustic displays of crickets. Finally, most studies evaluate the relationship between song parameters and morpho-physiological traits, but little is known about how individual motivation may affect the pattern of sound emission.

In the present study, we describe the aggressive song of *Melanotes ornata* Desutter-Grandcolas, 1993 (Phalangopsidae: Luzarinae) and evaluate what sort of information aggressive acoustic displays contain in order to understand their function in contests. *Melanotes ornata* is a nocturnal cricket species that is present in various microhabitats but is most commonly found on standing three trunks, large rocks close to watercourses or walking on the litter. These crickets occupy natural shelters like cavities on the trees' suber or beneath leafs that surround these trunks and males actively defended their calling sites against the approximation of conspecific males (G. Lobregat, personal observation). Their fight present two distinct phases (Lobregat et al., in prep.). In the first phase, individuals antennate and walk around each other gradually positioning their posterior region towards the opponent. When fights escalate to the second phase, individuals with their posterior region towards each other cross their hind legs, promoting pushes and kicks on the opponent with these legs, intermittently emitting aggressive acoustic signals of variable duration. The chance of contests escalate to the second phase is much higher when contestants have similar body sizes, and so, the emission of aggressive song usually occur in contests between size-matched contastants (Lobregat et al., in prep.) In this work, we evaluate if the aggressive song of *M. ornata* provide cues about fighting ability or motivational state. We manipulated contestants' motivation through controlled female offering before the fights and staged contests between males to record their aggressive song and evaluate if spectral, temporal parameters and signaling effort (time spent signaling) of the aggressive song are associated to body size (i.e. fighting ability) and motivation.

METHODS

Laboratory rearing

We captured *M. ornata* adult individuals in Estação Biológica de Santa Lúcia, municipality of Santa Teresa, Espírito Santo, Brazil in October and November 2016. During all experiment, individuals were housed in an environmentally controlled room with 12:12 h light/dark cycle, 25±2 ºC and 70±15% relative humidity. We established groups of one male and three females, which were housed in plastic buckets of 15cm diameter X 20cm height, where they were allowed to mate freely until their death. Females laid their eggs in cotton balls, and we grouped these cotton balls in a single plastic bucket, which we monitored weekly to detect hatching. We moved first instar nymphs to plastic containers of 50cm x 30cm x 30cm, and raised them until their adulthood. In the pre-adult stage, we individualized nymphs in plastic buckets of 10cm diameter X 15cm height to avoid interactions between adults. In this way, we ensured no effect of previous mating or agonistic experience between individuals (Iwasaki et al., 2006; Judge et al., 2010). In order to raise the total number of individuals and achieve an adequate number of observations for all experiments, we also captured wild nymphs of second and third stages in June and July 2017, at the same location as previously described. These wild nymphs were also raised in the laboratory until adulthood in the same way of laboratory nymphs. We provided humidified cotton, *ad libtum* fish food and egg cartoon as shelter to all containers where individuals were housed.

Obtainment of size-matched individuals

As already stated, the probability of a *Melanotes ornata* contest escalate to the second phase, in which individuals emit aggressive song, is higher when contestants are size-matched (Lobregat et al., in prep.). Thus, we measured pronotum length of individuals prior to the observations and paired size-matched individuals for each contest. For this, we exposed them individually to CO_2 in a plastic bag for 10 seconds, placed them on a millimeter paper and photographed with macro lens coupled to a Nikon D330 camera, which we positioned at a 0º angle in relation to individuals' body orientation and with a distance of 20 cm from them. We obtained pronotum length measures by importing photographs to ImageJ software and formed pairs of individuals with pronotum length difference less than 10mm. We took this photograph procedure in order to minimize handling and potential stress or damage to individuals, instead of taking direct measures with a caliper. However, we used pronotum length only to pair individuals with similar size. After contest observations (see section below), we euthanized individuals in alcohol 70% and measured their left hind femur length with a digital caliper, as such trait was already demonstrated as a proxy of fighting ability (Lobregat et al., in prep.)

Fight observations

We staged contests between males in an arena of 20cm diameter x 10cm height, with its walls covered with a black plastic and with a removable opaque divider in the center, forming two chambers in which individuals were allowed to acclimate for 10 minutes before fighting. *Melanotes ornata* males rarely show aggressive behavior in laboratory without female stimulus (G. Lobregat, personal observation). Thus, the opaque divider presented a two-chamber cage (3.5 cm diameter x 5 cm height) in its middle, where we placed a female in each chamber. The cage was perforated in order to allow antennal touch, but prevent mating between males in the arena and the female in the chamber. We used 12 females for this procedure, and for each day of observation, we previously randomized which pair of females would be used for each contest. We never placed the same female for different contest observations in the same day. After each contest observation, we cleaned the arena with 80% ethanol to avoid possible chemical cues in subsequent observations. We settled contests between 6 p.m. and 4 a.m. in a temperature-controlled room, with variation of 19.5 to 20.5ºC, to avoid fine-scale temperature effects on temporal components of song (Walker, 1962), and with red-dim light, once this species present nocturnal habit.

To stimulate individuals' motivation, we presented males to three females for two hours prior to contest observation, where males were allowed to touch females' antennae, but were prevented of mating with them. We did this by placing one male and three females in a plastic box of 12 cm width x 12 cm length x 3 cm height, with a perforated divider between male's and females' chamber, and an opaque divider between females' dividers. We used 18 females in this procedure, and for each day of observation, we previously randomized which group of females would be used for each contest. Not motivated males were placed in the same plastic box for 2 hours prior to contest observations, but with no females inside it. We never used a female for more than one observation within a day. After using a box, we always cleaned it with 80% ethanol in order to avoid chemical cues from previous individuals. We only registered contests where both males presented courtship behavior towards the female in the divider chamber during the 10 minutes of acclimation. We staged 15 contests between two motivated males and 16 contests between two non-motivated males and registered the second phase duration and total time with individuals crossing hind legs for each observation. However, for two observations in the motivated treatment, one of the males presented courtship behavior towards the other male, so we removed these observations from the analysis. We did not staged motivational asymmetric contests because such fights could end quickly due to other behaviors (e.g. kicking frequency) that could be more influenced by motivation than acoustic signaling. Thus, non-motivated males could flee from the contest before motivated males emitted the actual signaling effort motivation could confer to them. After contests, we euthanized individuals in alcohol 70% and measured hind femur length with an electronic caliper.

To register aggressive song of individuals, we placed a Sennheiser M6/K66 microphone, coupled with a SONY PCM-D50 audio recorder, in the top of the arena with an angle of 75º in relation to the arena floor, which we configured to record at a sampling rate of 22,050 Hz, at 16bit. This way, for each contest we had a single audio file containing all aggressive signals emitted along the observation. We also registered contests with a Nikon D3300 camera placed in the top of the arena, in order to allow the identification of which individual was singing in any particular time, by comparing audio and video registers.

Sound analysis of aggressive song

We analyzed sound files of each contest with Avisoft-SASLab Lite software, where we applied a Fast Fourier Transformation of 256 points, window FlatTop, frame size of 100% and overlap of 50%. Sometimes, the aggressive songs of different contestants were difficult to discriminate in the sound file as their sound frequencies were too similar. Thus, we compared video and audio files in order to detect what individual was emitting sound in any particular time in the audio file. *Melanotes ornata* aggressive signal present two distinct portions (Figure 1): a *trill* with constant emission of pulses (i.e. tegmen closure producing sound), which is mainly emitted when individuals are crossing their hind legs, and a *chirp* with regularly spaced groups of two pulses, which is mainly emitted when individuals are distant of each other (G. Lobregat, personal observation). These distinct portions have variable duration and their emission is associated to different circumstances in escalated contests, but such circumstances does not necessarily are followed by sound emission. We analyzed for each individual the following sound parameters: peak frequency (Hz), frequency bandwidth (Hz) and pulse rate (number of pulses per second) of the *trill* and the *chirp* portions (Brown et al., 2006; Walker, 1962). We measured the signaling effort of an individual as its total time emitting aggressive song along the contest, and also separated signaling effort of *trill* and *chirp* patterns of emission.

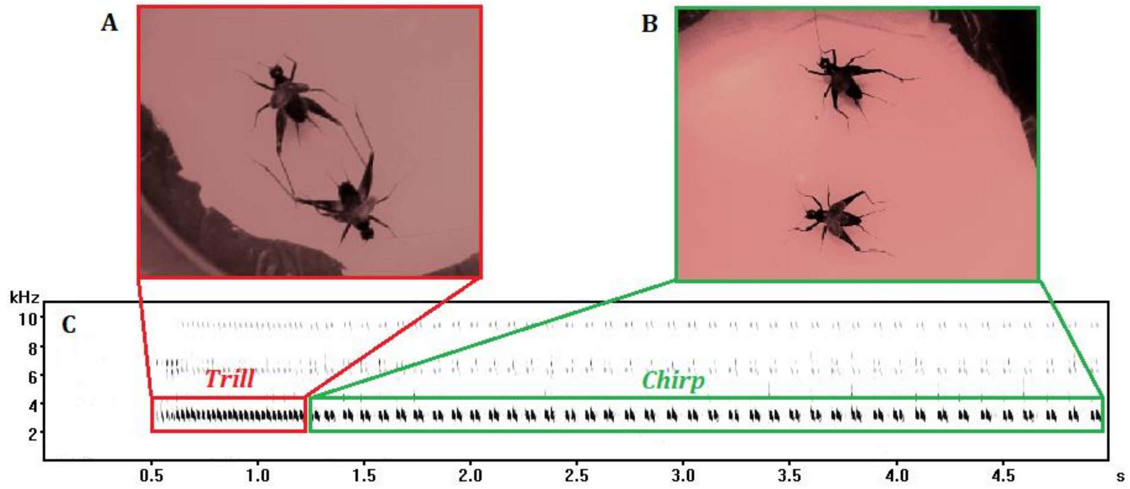

Figure 1. Aggressive acoustic signaling behavior of *Melanotes ornata* males. (A) Photograph of males emitting acoustic signals while crossing their hind legs in an escalated contest; (B) Photograph of males emitting acoustic signals while distant of each other; (C) Spectogram of a 5 seconds aggressive song section showing in red a *trill* pattern of sound emission and in green a *chirp* pattern of sound emission. The *trill* pattern of sound emission generally is present when individuals are crossing their hind legs, while *the* chirp pattern generally is present when individuals get distance of each other.

To obtain frequency measures, we chose a single pulse to which we applied a Power Spectrum analysis, and measured peak frequency as the frequency value with the highest intensity and frequency bandwidth as the frequency interval value at -20dB from peak frequency. To obtain pulse rate measures, we selected in the audio file three time intervals (ranging from 0.3 to one second) with *trill* emission and another three with *chirp* emission for each contestant. For each type of emission, the selected time intervals that presented at least 10 seconds between them along the sound recording. We calculated the pulse rate for each time interval and extracted their mean. Some individuals did not stridulate for more than 10 or 20 seconds, so for them we obtained the pulse rate from a single time interval or the mean pulse rate from two time intervals, respectively.

Statistical analysis

Signals must be consistent throughout the entire contest in order to present confident information about fighting ability (Brown et al., 2006). We used the package "rptR" to estimate repeatability of each sound parameter by calculating, for each individual, the intra-class correlation coefficient (ICC) between two measures of that sound parameter. This analysis describes the relative partitioning of variance into within-group and between-group sources of variance. In our particular case, within-group variation is considered as the difference between two measures of an individual's sound component, and between-group variation as the total range of values observed for that sound component for all individuals. High values of ICC indicate that variation within-group is lower than between-group, suggesting that a sound component is repeatable along the sound emission of an individual. We performed permutation tests by randomizing observed ICC 1000 times and estimating the probability of the observed data to be found under the null hypothesis calculated by the randomized data. We only used sound components that were repeatable in the posterior analyses.

To evaluate if aggressive signals contain information about fighting ability and motivation in their sound parameters, we adjusted different linear mixed models (LMMs) where different sound parameters were considered as the response variables in separate models and hind femur length (fighting ability proxy) or motivation treatment (motivated vs. non-motivated) were considered as the explanatory variables. We included an interaction term of femur length and motivation treatment, and added the contest in which each individual was observed as random intercept. These models were adjusted with Gaussian distribution of errors. We tested significance of femur length, motivation and their interaction with likelihood ratio tests (LRT).

To evaluate if signaling effort is associated to fighting ability and motivation, we used four composite measures of signaling effort as response variables: 1) the proportion of time with *trill* emission over the total time where contestants were crossing legs; 2) the proportion of time with *trill* emission over the duration of the escalated contest phase; 3) the proportion of time with *chirp* emission over the duration of the escalated contest phase and 4) the proportion of total time emitting signals (*trill* and *chirp*) over the duration of the escalated contest phase. We took this approach instead of using absolute values of total time signaling, since the overall duration of escalated contests may differ between motivated and non-motivated pairings, and a significant difference could be associated to different contest durations and not on how much an individual spent signaling along its contest. For each composite measure, we adjusted generalized linear mixed models (GLMM) where such measure was considered as the response variable and hind femur length, motivation treatment and their interaction as response variables. We also added the contest observation as a random intercept. We adjusted these models with Beta distribution of errors, which is recommended for response variables that are inherently proportions and not a proportion of the number of success and failures over the total sum of events, for which the binomial distribution would be recommended (Ferrari & Cribari-Neto, 2004). All statistical analyses were performed with R (R Core Team, 2018).

RESULTS

Sound parameters

Aggressive signals were highly repeatable, with peak frequency, frequency bandwidth and *chirp* pulse rate presenting a significant ICC (Table 1). The only sound parameter that was not significantly repeatable was the t*rill* pulse rate, so we did not evaluate its relationship with hind femur size or motivation. Hind femur length had no relationship with peak frequency ($\chi^2 = 0.27$; n = 43; p = 0.59), frequency bandwidth ($\chi^2 = 0.09$; n = 41; p = 0.62) or *chirp* pulse rate ($\chi^2 = 0.003$, n = 31; p = 0.95). *Chirp* pulse rate was greater for motivated individuals in comparison to non-motivated ones ($\chi^2 = 5.22$; n = 38; p = 0.02), whereas peak frequency ($\chi^2 = 3.32$; n = 43; p = 0.07) and frequency bandwidth ($\chi^2 = 1.34$, n = 43; p = 0.29) had no relationship with motivation treatment. Neither interaction terms were significant (p > 0.17).

Table 1. Results of repeatability analysis for each parameter of aggressive signals of *Melanotas ornata*. Confidence intervals (C.I.) were calculated from bootstrapping (n=1000 iterations).

Sound parameter	**ICC**	**SE**	**C.I.**	**Number of songs analysed**	**Permutation test p-value**
Peak frequency	0.982	0.007	0.963 – 0.991	29	0.001
Frequency Bandwidth	0.631	0.122	0.337 – 0.817	28	0.001
Trill pulse rate	0	0.166	0 – 0.551	11	1
Chirp pulse rate	0.623	0.131	0.31 – 0.813	25	0.001

ICC: Infra-class correlation coefficient (repeatability index).

Signalling effort

Overall, motivated pairs spent more time emitting aggressive signals than non-motivated pairs (Table 2). Such effect of motivation on total acoustic signals emission was most prominent when comparing the proportion of time with *trill* emission when individuals were crossing legs, where motivated pairings presented a mean proportion of 0.46, almost three times higher than non-motivated pairings with a mean proportion of 0.17. The proportion of time with *trill* emission while individuals were crossing legs was higher when contestants were motivated than non-motivated pairs ($\chi^2_1 = 14.43$; n = 58; p <0.001; Fig. 2A). In the same way, the proportion of time with *trill* emission in the escalated phase was higher in the motivated treatment ($\chi^2_1 = 12.59$; n = 58; p <0.001; Fig. 2B). When considering *chirp* emission, motivated individuals also spent a higher proportion of the escalated phase duration emitting aggressive signals ($\chi^2_1 = 8.73$; n = 58; p =0.003; Fig. 2C). Considering total time emitting signals, regardless of which type of signal, motivated pairs also spent a higher proportion of time in the escalated phase emitting acoustic signals ($\chi^2_1 = 11.81$; n = 58; p <0.001; Fig. 2D). Neither interaction terms were significant (p >0.14).

Table 2. Comparison of composite measurements of time spent emitting signalling aggressive song between motivated and non-motivated pairings of *Melanotes ornata* contests.

Measurement	**Motivated pairings**			**Non-motivated pairings**		
	Mean	**Minimum**	**Maximum**	**Mean**	**Minimum**	**Maximum**
Proportion of time emitting trill while crossing legs	0.459	0.103	0.959	0.168	0	0.593
Proportion of time emitting trill during the second phase	0.222	0.07	0.662	0.093	0	0.404
Proportion of time emitting chirp during the second phase	0.407	0.163	0.691	0.283	0	0.839
Proportion of time emitting signals during the second phase	0.628	0.265	1	0.377	0	0.96

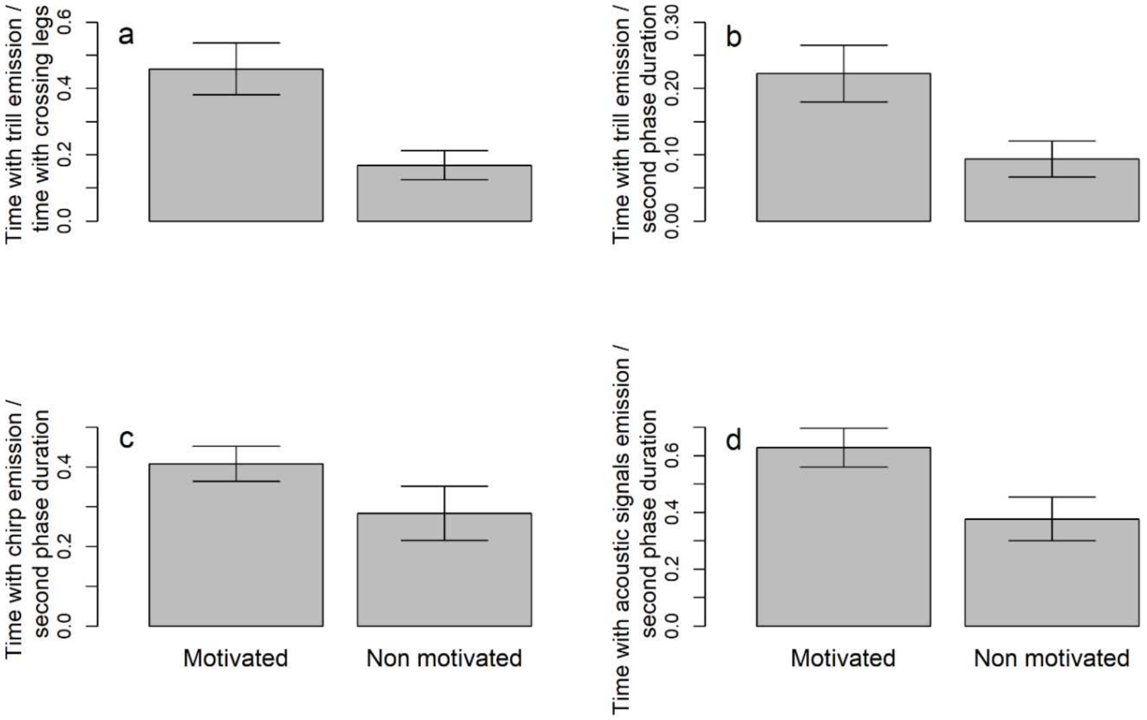

Figure 2. Difference in time spent signaling aggressive song between motivated and non-motivated pairings of *Melanotes ornata* males. (a) Proportion of time with *trill* emission while individuals were crossing legs; (b) Proportion of time with *trill* emission during the second phase; c) Proportion of time with *chirp* emission during the second phase; (d) Proportion of time with acoustic signals (*trill* and *chirp*) emission during the second phase. Horizontal bars represent the standard deviation. All relationships were significant (p<0.01).

DISCUSSION

In this work, we demonstrated that motivated pairings of *M. ornata* male contestants emitted the aggressive song for a longer duration than non-motivated pairings and that *chirp* pulse rate is greater for motivated individuals, suggesting that temporal fine-scale parameters as well as the signaling effort of the aggressive song carry significant information about individual motivation in this species. On the other hand, the temporal and spectral sound parameters of aggressive song here investigated had no relationship with hind femur length of *M. ornata* individuals. Since lengthier hind legs are associated to higher changes of winning in this species (Lobregat et al., in prep.), our results show that the aggressive song does not signalize individuals' fighting ability, but has an important role in signaling motivation to the opponent along escalated contests. Game theory suggests that if contestants have similar fighting abilities, motivation is the main factor determining the chances of winning, as motivated individuals are willing to pay a greater amount of costs in the fight (Enquist & Leimar, 1987; Hammerstein & Parker, 1982; Leimar & Enquist, 1984). In *M. ornata* fights, the emission of aggressive sound signals is restricted to escalated contests, which typically occur when contestants present very similar fighting abilities (Lobregat et al., in prep.). In such escalated contests, it is possible that the small differences in fighting ability have a minor role in the contest result than differences in motivation. Thus, signaling motivation instead of signaling fighting ability would be more advantageous, as it would accelerate the loser's decision to retreat in a greater extent than signaling fighting ability.

Evidences in a wide array of species demonstrate that contestants adjust their agonistic behavior according to the value of the contested resource (Gareth Arnott & Elwood, 2008), for example, by displaying agonistic signals at higher intensity when motivated (Arnott & Elwood, 2007; Hofmann & Schildberger, 2001; Kotiaho et al, 1999; Ripmeester et al., 2007; Yack et al., 2014). By manipulating the perceived resource value in contests of *M. ornata* males, we showed that individuals with longer prior contact with potential mates, but incapable to mate with them, presented higher signaling effort and higher pulse rate in their acoustic displays. Our results contrast with the only study, to our knowledge, that evaluated the influence of motivation on the aggressive song of crickets. Instead of information about motivation, fine-scale temporal parameters of the aggressive song of the house-cricket *Acheta domesticus* inform fighting ability (Brown et al., 2006). A possible explanation for this different function of aggressive song is the moment of the fight where individuals emit the aggressive song in these species. In *M. ornata*, aggressive sound signaling is restricted to escalated fights, where contestants usually present similar fighting abilities and motivation probably plays a greater role than fighting ability in the contest result. In contrast, most fights of house-crickets do not escalate beyond stridulation and mandible flaring (Adamo & Hoy, 1995; Hack, 1997a; Tachon et al., 1999), which are low-cost displays (Hack, 1997b), and could be used as initial tactics to inform fighting ability.

For signals to contain reliable information there must be some kind of cost or constraint associated to signaling that minimizes the chances of lower quality senders exaggerating their signal intensity (i.e. to bluff) (Vehrencamp, 2000; Zahavi & Zahavi, 1999). We suggest that one potentially major cost of sound signaling along the contests of *M. ornata* is an increased predation risk of sound-guided natural enemies. In crickets, several cases have been demonstrated where predators and parasitoids use the victims' acoustic cues to locate their prey (review in Zuk & Kolluru, 1998). In the particular case of aggressive song emission, there may be an additional challenge, as far as signaling individuals must divide their attention between the actions of the fighting rival and defending themselves of predators attack during escalated fights (Jakobsson, Brick, & Kullberg, 1995). Thus, as predator or parasitoid exposure increases with acoustic signaling, it is expected that individuals in higher motivational state may present a higher signaling effort as they are willing to pay higher costs in the fight. Such effect of motivation on predator exposure was demonstrated in contests of territorial lizards *Tropidurus hispidus* (Diaz-Uriarte, 1999). In this study, territory owners that previously fought against intruders exposed themselves more to predators attack in their territory than individuals that did not have defended their territory previously, suggesting that individuals balance the costs of predation risk and the benefits of territory holding. We suggest that such balance of costs and benefits may occur in *M. ornata* males, where motivated individuals present higher aggressive acoustic signaling effort as they are willing to expose themselves to a higher predation risk by sound-guided natural enemies, causing less motivated individuals to flee sooner.

Other possible mechanisms of bluffing control are the handicap between signal intensity and an energetic cost associated to signal production, or physical constraints associated to the size and form of the signal producing structures (Grafen, 1990; Smith & Harper, 1995). In various gryllinae crickets, acoustic signaling seems to be energetically demanding (Kavanagh, 1987; Prestwich & Walker, 1981), and energy consumption while stridulating appears to be associated to wing stroke rate in crickets (Prestwich & Walker, 1981). If such relationship holds for *M. ornata* sound production, the positive effect of motivation on *chirp* pulse rate and time spent signaling suggests that motivated individuals are willing to expend more energy in sound emission, informing their rivals a higher motivational state. However, evidences that sound production is energy expensive in crickets are exclusively associated to mating signals and the only study that investigated the energetic cost of aggressive signals demonstrated that sound emission was one of the cheapest activities of the agonistic repertoire (Hack, 1997b). Regarding the physical constraints associated to sound production, crickets are incapable of modulating their sound frequencies in a great extent due to the resonance properties of their forewings (Koch et al., 1988). There is usually a negative correlation between the size of sound-producing structures and the sound frequency they emit (Moradian & Walker, 2008), and if the size of such structures correlates to the overall body size of individuals, the sound frequency may be a reliable index of fighting ability (Simons, 1995). Contrary to these predictions, we did not find a correlation between spectral components of aggressive song and size of *M. ornata* males. We argue that, although a relationship between wing size and sound frequency may be true, the relationship between overall size and wing size of individuals could be weak for this species, with some small individuals with larger wings than large males. However, sound frequency still may provide other sort of information if the development of the wings is associated to past condition in earlier developmental stages (H. Scheuber et al., 2003).

In conclusion, our study demonstrated that the aggressive sound of *M. ornata* contains information on individuals' motivation, while fighting ability is probably accessed by different means like other agonistic tactics or prior to contest escalation in this species (see Lobregat et al., in prep.). This is, to our knowledge, the first clear evidence of motivation content in aggressive sound signals produced by crickets along fights. Receivers' prospects of winning the contest may be hampered when facing a highly motivated rival. Alternatively, such acoustic signals may increase the chances of attracting a predator. Thus, high intensity signals displayed by motivated individuals may potentially affect the decision of receivers to stay in the fight, accelerating the less motivated individual retreat.

REFERENCES

Adamo, S. A., & Hoy, R. R. (1995). Agonistic behaviour in male and female field crickets, *Gryllus bimaculatus*, and how behavioural context influences its expression. Animal Behaviour, 49(6), 1491–1501.

Alexander, R. D. (1961). Aggressiveness, territoriality, and sexual behavior in field crickets (Orthoptera: Gryllidae). Behaviour, 17(2), 130–223.

Alexander, R. D. (1962). Evolutionary Change in Cricket Acoustical Communication. Evolution, 16(4), 443–467.

Arnott, G., & Elwood, R. W. (2007). Fighting for shells: how private information about resource value changes hermit crab pre-fight displays and escalated fight behaviour. Proceedings of the Royal Society B: Biological Sciences, 274(1628), 3011–3017.

Arnott, G., & Elwood, R. W. (2008). Information gathering and decision making about resource value in animal contests. Animal Behaviour, 76(3), 529–542.

Bertram, S. M., & Rook, V. (2012). Relationship between condition, aggression, signaling, courtship, and egg laying in the field cricket, *Gryllus assimilis*. Ethology, 118(4), 360–372.

Brown, W. D., Smith, A. T., Moskalik, B., & Gabriel, J. (2006). Aggressive contests in house crickets: size, motivation and the information content of aggressive songs. Animal Behaviour, 72(1), 225–233.

Cade, W. H. (1984). Effects of fly parasitoids on nightly calling duration in field crickets. Canadian Journal of Zoology, (62), 226-228.

Clutton-Brock, T. H., & Albon, S. D. (1979). The roaring of red deer and the evolution of honest advertisement. Behaviour, 69(3–4), 145–170.

Diaz-Uriarte, R. (1999). Anti-predator behaviour changes following an aggressive encounter in the lizard *Tropidurus hispidus*. Proceedings of the Royal Society B: Biological Sciences, 266(1437), 2457–2464.

Eberhardt, L. S. (1994). Oxygen consumption during singing by male carolina wrens (*Thryothorus ludovicianus*). Auk, 111(1), 124–130.

Enquist, M., & Leimar, O. (1983). Evolution of fighting behaviour: Decision rules and assessment of relative strength. Journal of Theoretical Biology, 102(3), 387–410.

Enquist, M., & Leimar, O. (1987). Evolution of fighting behaviour: The effect of variation in resource value. Journal of Theoretical Biology, 127(2), 187–205.

Ferrari, S. L. P., & Cribari-Neto, F. (2004). Beta regression for modelling rates and proportions. Journal of Applied Statistics, 31(7), 799–815.

Grafen, A. (1990). Biological signals as handicaps. Journal of Theoretical Biology, 144(4), 517–546.

Gray, D. A. (1997). Female house crickets,*Acheta domesticus*, prefer the chirps of large males. Animal Behaviour, 54(6), 1553–1562.

Hack, M. A. (1997a). Assessment strategies in the contests of male crickets, *Acheta domesticus* (L.). Animal Behaviour, 53, 733–747.

Hack, M. A. (1997b). The energetic costs of fighting in the house cricket, *Acheta domesticus* L. Behavioral Ecology, 8(1), 28–36.

Hammerstein, P., & Parker, G. A. (1982). The asymmetric war of attrition. Journal of Theoretical Biology, 96(4), 647–682.

Hedrick, A. (2005). Environmental condition-dependent effects on a heritable, preferred male trait. Animal Behaviour, 70(5), 1121–1124.

Hofmann, H. A., & Schildberger, K. (2001). Assessment of strength and willingness to fight during aggressive encounters in crickets. Animal Behaviour, 62, 337–348.

Holzer, B., Jacot, A., & Brinkhof, M. W. G. (2003). Condition-dependent signaling affects male sexual attractiveness in field crickets, *Gryllus campestris*. Behavioral Ecology, 14(3), 353–359.

Hurd, P. L. (1997). Is signalling of fighting ability costlier for weaker individuals? Journal of Theoretical Biology, 184(1), 83–88.

Iwasaki, M., Delago, A., Nishino, H., & Aonuma, H. (2006). Effects of previous experience on the agonistic behaviour of male crickets, *Gryllus bimaculatus*. Zoological Science, 23(10), 863–872.

Jacot, A., Scheuber, H., & Brinkhof, M. W. G. (2010). Costs of an Induced Immune Response on Sexual Display and Longevity in Field Crickets. Evolution, 58(10), 2280–2286.

Jakobsson, S., Brick, O., & Kullberg, C. (1995). Escalated fighting behaviour incurs increased predation risk. Animal Behaviour, 49(1), 235–239.

Johnstone, R. A. (1995). Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. Biological Reviews, 70(1), 1–65.

Judge, K. A. (2011). Do male field crickets, *Gryllus pennsylvanicus*, signal their age? Animal Behaviour, 81(1), 185–194.

Judge, K. A., Ting, J. J., & Gwynne, D. T. (2008). Condition dependence of male life span and calling effort in a field cricket. Evolution, 62(4), 868–878.

Judge, K. A., Ting, J. J., Schneider, J., & Fitzpatrick, M. J. (2010). A lover, not a fighter: Mating causes male crickets to lose fights. Behavioral Ecology and Sociobiology, 64(12), 1971–1979.

Kavanagh, M. W. (1987). The efficiency of sound production in two cricket Species, *Gryllotalpa australis* and *Teleogryllus commodus* (Orthoptera, Grylloidea). Journal of Experimental Biology, 130, 107–119.

Koch, U. T., Elliott, C. J. H., Schäffner, K. H., & Kleindienst, H. U. (1988). The mechanics of stridulation of the cricket *Gryllus campestris*. Journal of Comparative Physiology A, 162(2), 213–223.

Kotiaho, J. S., Alatalo, R. V., Mappes, J., & Parri, S. (1999). Honesty of agonistic signalling and effects of size and motivation asymmetry in contests. Acta Ethologica, 2(1), 13–21.

Leimar, O., & Enquist, M. (1984). Effects of asymmetries in owner-intruder conflicts. Journal of Theoretical Biology, 111(3), 475–491.

Leonard, A. S., & Hedrick, A. V. (2010). Long-distance signals influence assessment of close range mating displays in the field cricket, *Gryllus integer*. Biological Journal of the Linnean Society, 100(4), 856–865.

Maynard-Smith, J., & Parker, G. A. (1976). The logic of asymmetric animal contests. Animal Behaviour, 24, 159–175.

Mesterton-Gibbons, M., Marden, J. H., & Dugatkin, L. A. (1996). On wars of attrition without assessment. Journal of Theoretical Biology, 181(1), 65–83.

Moradian, N. R., & Walker, S. E. (2008). Relationships between body size and sound-producing structures in crickets: Do large males have large harps? Invertebrate Biology, 127(4), 444–451.

Mowles, S. L. (2014). The physiological cost of courtship: Field cricket song results in anaerobic metabolism. Animal Behaviour, 89, 39–43.

Orozco, S. X., & Bertram, S. M. (2004). Parasitized male field crickets exhibit reduced trilling bout rates and durations. Ethology, 110(11), 909–917.

Payne, R. J. H., & Pagel, M. (1996). When is false modesty a false economy? An optimality model of escalating signals. Proceedings of the Royal Society B: Biological Sciences, 263, 1545–1550.

Payne, R. J. H., & Pagel, M. (1997). Why do animals repeat displays? Animal Behaviour, 54(1), 109–119.

Prestwich, K. N., & Walker, T. J. (1981). Energetics of singing crickets: Effects of temperature in three trilling species (Orthoptera:Gryllidae). Journal of Comparative Physiology, 143, 199–212.

R Core Team. (2016). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Rantala, M. J., & Kortet, R. (2003). Courtship song and immune function in the field cricket *Gryllus bimaculatus*. Biological Journal of the Linnean Society, 79(3), 503–510.

Reichert, M. S. (2013). Sources of variability in advertisement and aggressive calling in competitive interactions in the grey treefrog, *Hyla versicolor*. Bioacoustics, 22(3), 195–214.

Reichert, M. S. (2014). Playback tests and studies of animal contest dynamics: Concepts and an example in the gray tree frog. Behavioral Ecology, 25(3), 591–603.

Ripmeester, E. A. P., De Vries, A. M., & Slabbekoorn, H. (2007). Do blackbirds signal motivation to fight with their song? Ethology, 113(11), 1021–1028.

Ryan, M. J. (1988). Constraints and patterns in the evolution of anuran acoustic communication. In B. Fritzsch, T. E. Hetherington, M. J. Ryan, W. Walkowiak, & W. Wilczynski (Eds.), The Evolution of the amphibian auditory system (pp. 637–677). New York: Wiley.

Scheuber, H., Jacot, A., & Brinkhof, M. W. G. (2003). Condition dependence of a multicomponent sexual signal in the field cricket *Gryllus campestris*. Animal Behaviour, 65(4), 721–727.

Scheuber, H., Jacot, A., & Brinkhof, M. W. G. (2003). The effect of past condition on a multicomponent sexual signal. Proceedings of the Royal Society B: Biological Sciences, 270(1526), 1779–1784.

Searcy, W. A., & Andersson, M. (1986). Sexual selection and the evolution of song. Annual Review of Ecology and Systematics, 17(May), 507–533.

Simmons, L. W., & Zuk, M. (1992). Variability in call structure and pairing success of male field crickets, *Gryllus bimaculatus*: the effects of age, size and parasite load. Animal Behaviour, 44(6), 1145–1152.

Simons, L. W. (1995). Correlates of male quality in the fiels cricket, *Gryllus campestris* L.: age, size, and summetry determine pairing success in field populations. Behavioral Ecology, 6(4), 376–381.

Smith, M. J., & Harper, D. G. C. (1995). Animal signals: Models and terminology. Journal of Theoretical Biology, 177(3), 305–311.

Tachon, G., Murray, A., Gray, D. A., & Cade, W. H. (1999). Agonistic displays and the benefits of fighting in the field cricket, *Gryllus bimaculatus*. Journal of Insect Behavior, 12(4), 533–543.

Tregenza, T., Simmons, L. W., Wedell, N., & Zuk, M. (2006). Female preference for male courtship song and its role as a signal of immune function and condition. Animal Behaviour, 72(4), 809–818.

Vehrencamp, S. L. (2000). Handicap, index, and conventional signal elements of bird song. In: Espmark, Y., Amundsen, T., Rosenqvist, G. (eds). Animal Signals: Signalling and Signal Design in Animal Communication, 277–300.

Wagner, W. E., & Hoback, W. W. (1999). Nutritional effects on male calling behaviour in the variable field cricket. Animal Behaviour, 57(1), 89–95.

Walker, T. J. (1962). Factors responsible for intraspecific variation in the calling songs of crickets. Evolution, 16(4), 407-428.

Wyman, M. T., Mooring, M. S., McCowan, B., Penedo, M. C. T., & Hart, L. A. (2008). Amplitude of bison bellows reflects male quality, physical condition and motivation. Animal Behaviour, 76(5), 1625–1639.

Yack, J. E., Gill, S., Drummond-Main, C., & Sherratt, T. N. (2014). Residency duration and shelter quality influence vibratory signalling displays in a territorial caterpillar. Ethology, 120(4), 354–364.

Zahavi, A. (1975). Mate selection-A selection for a handicap. Journal of Theoretical Biology, 53(1), 205–214.

Zahavi, A., & Zahavi, A. (1999). The handicap principle: A missing piece of Darwin's puzzle. New York: Oxford University Press.

Zuk, M., & Kolluru, G. R. (2010). Exploitation of sexual signals by predators and parasitoids. The Quarterly Review of Biology, 73(4), 415–438.

GENERAL CONCLUSIONS

Based on the results presented in the three chapters, we present the following conclusions:

1) Contests following cumulative assessment strategy may present relationships between contest duration and body size similar to what is predicted by pure self-assessment, mutual assessment and opponent-only assessment. Thus, in injurious contests, it is important to evaluate the offensive capacity of contestants, either by a direct measure of damage imposition and its relationship with body size, or by the evaluation of the allometric relationship between offensive and defensive attributes.
2) When contests follow different assessment strategies in their behavioral phases, the evaluation of overall contest duration alone hinders the determination of what assessment strategy is applied in escalated phases. As many studies have found evidences for more than one model, but few have explicitly tested the relationship between the duration of different phases and body size, switching assessment may be more general than was previously thought.
3) The contest behavior of *Melanotes ornata* gives empirical support for the second conclusion. In the first contest phase, individuals mutually assess each other and the probability of fights escalate to the second phase is much higher when individuals have similar fighting abilities. However, in the second phase we did not find evidences of fighting ability assessment. We suggest that other asymmetries may be more important in the contest resolution in escalated fights, like differences in motivational status.
4) The aggressive song emitted along escalated phases of *M. ornata* present features that are correlated to the motivational status of the senders. Motivated males exhibit

a higher signaling effort and their acoustic signals present higher *chirp* pulse rates. Thus, contestants may use this information contained in such displays to help in their decision to stay or leave the contest.

SUPPLEMENTARY MATERIAL

Suplementary Material: How offensive capacity and switching assessment between contest stages can hinder empirical testing of fighting assessment models

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07-10-2018

1 Data simulation

1.1 Populations

Simulating one thousand populations of n=200 individuals, with a mean body size of 30 cm and a standard deviation of 6 cm.

```
> set.seed(1)
> nrSamples = 1000
> populationsize = list(mode="vector",length=nrSamples)
> for (i in 1:nrSamples) {
+   populationsize[[i]] = rnorm(200, mean = 30, sd = 6)
+ }
```

Simulating fighting ability by adding a standar deviation of 4 to the values of **populationsize**.

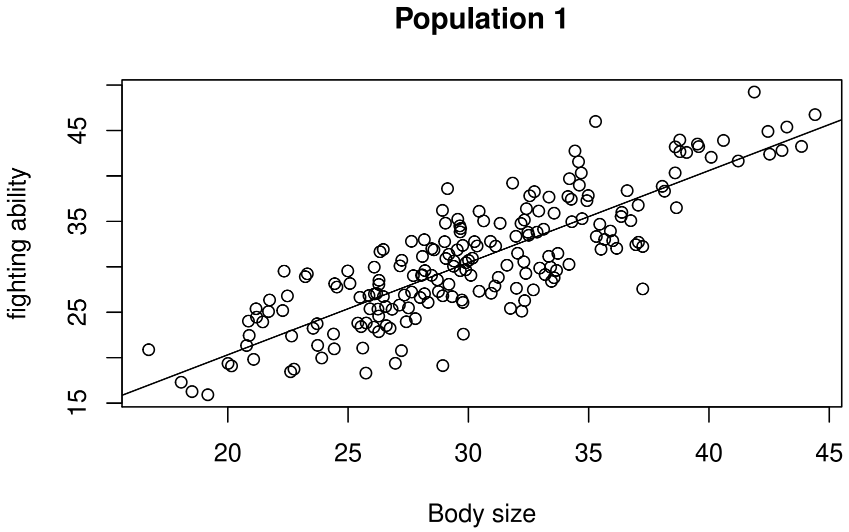
```
> populationrhp = list(mode="vector",length=nrSamples)
> for (i in 1:nrSamples) {
+   populationrhp[[i]] = populationsize[[i]]+rnorm(200,sd=4)
+ }
>
```

Comparing the mean and the standard deviation of body size and fighting ability in population 1.

```
> mean(populationsize[[1]])

[1] 30.21324

> sd(populationsize[[1]])

[1] 5.574584

> mean(populationrhp[[1]])
```

```
[1] 30.66849
> sd(populationrhp[[1]])
[1] 6.744757
>
```

Demonstrative of the relationship between body size and fighting ability in population 1.



1.2 Arranging pairings

First, I randomly pair individuals' fighting ability and body size values. By using the command "set.seed(1)", I ensure that the same randomization was applied for both vectors.

```
> set.seed(1)
> pairsrhp = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   pairsrhp[[i]] = matrix(sample(populationrhp[[i]]), ncol = 2)
+ }
> set.seed(1)
> pairssize = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   pairssize[[i]] = matrix(sample(populationsize[[i]]), ncol = 2)
+ }
> head(pairssize[[1]])
```

```
         [,1]     [,2]
[1,] 23.22382 31.13275
[2,] 22.47820 31.83935
[3,] 26.09582 40.09306
[4,] 36.16435 22.34045
[5,] 34.57905 34.27600
[6,] 29.79164 35.51386
```

This code generates for each simulated population, two objects representing the contests: pairsrhp, which reffers to the fighting ability values of contestants and pairssize, which reffers to the body size of contestants.

1.3 Obtaining vectors of fighting ability and body size of losers and winners, as well as their difference and mean values

First, we consider for each contest (i.e. each pair) that the individual with the lower fighting ability is the loser and vice versa.

```
> loserrhp= list(length=nrSamples)
> for (i in 1:nrSamples) {
+   loserrhp[[i]] = apply(pairsrhp[[i]],1,min)
+ }
> winnerrhp = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   winnerrhp[[i]] = apply(pairsrhp[[i]],1,max)
+ }
>
```

However, the same procedure should not be applied to body size. This is because individuals with lower fighting ability may present greater body size due to the standard deviation we applied before.

First, I create two data frames for each population, using **pairsrhp** and **pairssize**.

```
> dfpairsrhp = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   dfpairsrhp[[i]] = as.data.frame(pairsrhp[[i]])
+ }
> dfpairssize = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   dfpairssize[[i]] = as.data.frame(pairssize[[i]])
+ }
```

Then, I create a logical vector (TRUE or FALSE) indicating for each line of the pairsrhp dataframe if the first column value is greater than the second column. This logical vector simply indicate if the individual in the first column is the winner for that pair.

```
> rhpv1maior = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   rhpv1maior[[i]] = dfpairsrhp[[i]]$V1>dfpairsrhp[[i]]$V2
+ }
```

Next, I add this logical vector to the data frame of pairs size.

```
> dfpairssize1 = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   dfpairssize1[[i]] = data.frame(dfpairssize[[i]]$V1,dfpairssize[[i]]$
+                                     V2,rhpv1maior[[i]])
+ }
```

Finally, I use the function **ifelse** to determine for each pair in the pairssize which is the loser and which is the winner.

```
> losersize= list(length=nrSamples)
> for (i in 1:nrSamples) {
+   losersize[[i]] = ifelse(dfpairssize1[[i]]$rhpv1maior..i..==FALSE,
+                           dfpairssize1[[i]]$dfpairssize..i...V1,
+                           dfpairssize1[[i]]$dfpairssize..i...V2)
+ }
> winnersize= list(length=nrSamples)
> for (i in 1:nrSamples) {
+   winnersize[[i]] = ifelse(dfpairssize1[[i]]$rhpv1maior..i..==TRUE,
+                            dfpairssize1[[i]]$dfpairssize..i...V1,
+                            dfpairssize1[[i]]$dfpairssize..i...V2)
+ }
```

I also create vectors for the difference in fighting ability and body size of each pair, as well as the mean fighting ability and mean body size.

```
> zrhp = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   zrhp[[i]] = apply(pairsrhp[[i]],1,diff)
+ }
> diffrhp = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   diffrhp[[i]] = abs(zrhp[[i]])
+ }
> meanrhp = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   meanrhp[[i]] = apply(pairsrhp[[i]],1,mean)
+ }
> zsize = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   zsize[[i]] = apply(pairssize[[i]],1,diff)
+ }
> diffsize = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   diffsize[[i]] = abs(zsize[[i]])
+ }
> meansize = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   meansize[[i]] = apply(pairssize[[i]],1,mean)
+ }
```

1.4 Arranging *size-matched* contests

First, I create for each population a data frame containing all vectors constructed in the above section.

```
> datapops = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   datapops[[i]] = data.frame(losersize[[i]],winnersize[[i]],
+                              diffsize[[i]],meansize[[i]],loserrhp[[i]],
+                              winnerrhp[[i]],diffrhp[[i]],meanrhp[[i]])
+ }
```

Then, I create for each population a similarity threshold to be used as an indicative that a given pair is size-matched. This threshold is considered as 10% of the total variation in body size found for that population.

```
> minimum = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   minimum[[i]] = min(pairssize[[i]])
+ }
> maximum = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   maximum[[i]] = max(pairssize[[i]])
+ }
> similarthreshold = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   similarthreshold[[i]] = 0.1*(maximum[[i]]-minimum[[i]])
+ }
```

Finally, I create a subset of the created data frames where the difference in body size is smaller than such similarity threshold. This subset contains all size-matched contests for a population.

```
> datapopsmatch = list(length=nrSamples)
> for (i in 1:nrSamples) {
+   datapopsmatch[[i]] = subset(datapops[[i]],
+                               diffsize[[i]]<similarthreshold[[i]])
+ }
> match<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   match[[i]] <- as.data.frame(datapopsmatch[[i]])
+ }
>
```

2 Simulating different offensive capacities

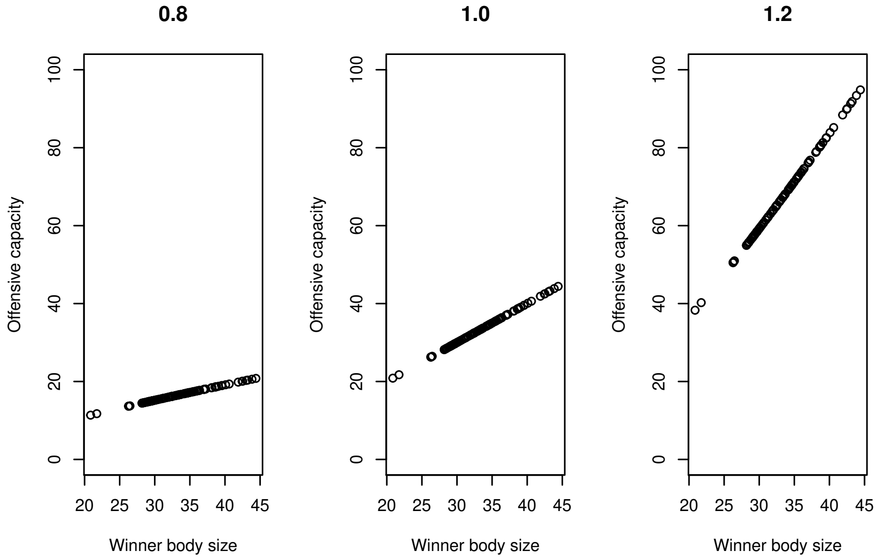
I simulated different offensive capacities by calculating damage as a function of winner body size with three exponents: 0.8 (low offensive capacity), 1(medium offensive capacity) and 1.2 (high offensive capacity).

I did this for both ramdom pairings and size-matched pairings.

```
> damage0.8<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   damage0.8[[i]] <- winnersize[[i]]^0.8
+ }
> damage1<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   damage1[[i]] <- winnersize[[i]]^1
+ }
> damage1.2<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   damage1.2[[i]] <- winnersize[[i]]^1.2
+ }
>
```

```
> damage0.8match<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   damage0.8match[[i]] <- match[[i]]$winnersize^0.8
+ }
> damage1match<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   damage1match[[i]] <- match[[i]]$winnersize^1
+ }
> damage1.2match<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   damage1.2match[[i]] <- match[[i]]$winnersize^1.2
+ }
```

The following plots demonstrate the relationship between winner body size and damage for the three scenarios in Population 1.

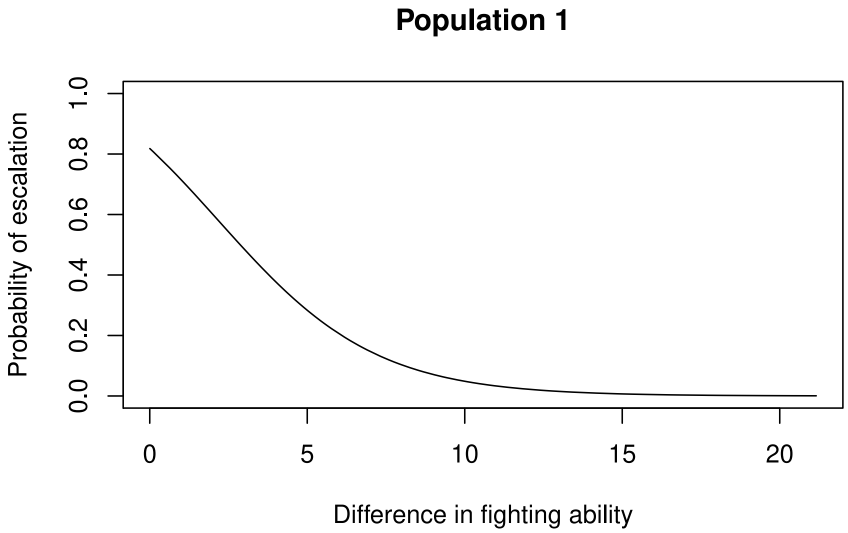


3 Determining the escalation rule for switching assessment contests

As not all contests are expected to escalate to the second phase, we calculated a probability function determining when a contest would have a higher probability of escalation. As we considered a scenario where fights escalate from mutual assessment to pure self-assessment, we used the escalation rules of mutual assessment. According to this model, fights should escalate when the difference in fighting ability is small. Thus, the probability of escalation was negatively determined by the difference in fighting ability between individuals.

```
> betaSAM = -0.4
> probescalationSAM<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   probescalationSAM[[i]] <- similarthreshold[[i]]/
+     (1+exp(-betaSAM*diffrhp[[i]])+similarthreshold[[i]]/2)
+ }
```

Probability function of escalation for Population 1.



Then, I created a binary vector for each population, indicating if a contest escalated (1) or did not escalated (0) to the next phase. I used the simulated probability function in a simulation following a binomial distribution.

```
> escalationSAM<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   escalationSAM[[i]] <- rbinom(100,1,prob=probescalationSAM[[i]])
+ }
>
```

4 Estimating duration by following assessment strategies predictions

For all pairings of each population, we estimated contest duration for each assessment strategy, following their predictions.

4.1 Pure self-assessment

```
> interceptWOA=0
> slopeWOA=1
> durationWOA= list(length=nrSamples)
> for (i in 1:nrSamples) {
+   durationWOA[[i]] = interceptWOA+slopeWOA*loserrhp[[i]]
+ }
>
```

4.2 Cumulative assessment

Here, we have three duration objects associated to different offensive capacities.

```
> interceptCAM=50
> slopeCAM1=1
> slopeCAM2_0.8<- list(length=nrSamples)
```

```
> for (i in 1:nrSamples) {
+   slopeCAM2_0.8[[i]] <- damage0.8[[i]]
+ }
> durationCAM0.8<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   durationCAM0.8[[i]] <- interceptCAM+slopeCAM1*loserrhp[[i]]
+   -slopeCAM2_0.8[[i]]
+ }
> slopeCAM2_1<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   slopeCAM2_1[[i]] <- damage1[[i]]
+ }
> durationCAM1<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   durationCAM1[[i]] <- interceptCAM+slopeCAM1*loserrhp[[i]]
+   -slopeCAM2_1[[i]]
+ }
> slopeCAM2_1.2<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   slopeCAM2_1.2[[i]] <- damage1.2[[i]]
+ }
> durationCAM1.2<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   durationCAM1.2[[i]] <- interceptCAM+slopeCAM1*loserrhp[[i]]
+   -slopeCAM2_1.2[[i]]
+ }
>
```

4.3 Mutual assessment

```
> interceptSAM=50
> slopeSAM=-1
> durationSAM= list(length=nrSamples)
> for (i in 1:nrSamples) {
+   durationSAM[[i]] = interceptSAM+slopeSAM*diffrhp[[i]]
+ }
>
```

4.4 Opponent-only assessment

```
> interceptOOA=50
> slopeOOA=-1
> durationOOA= list(length=nrSamples)
> for (i in 1:nrSamples) {
+   durationOOA[[i]] = interceptOOA+slopeOOA*winnerrhp[[i]]
+ }
>
```

4.5 Switching from mutual to pure self-assessment

Here, we calculated overall fight duration, the duration of the first phase (mutual assessment) and the duration of the second phase (pure self-assessment). Overall duration is equal to firstphase duration when fights do not escalate and is equal to the sum of first and second phase durations when fights escalates.

```
> durationphase1SAM<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   durationphase1SAM[[i]] <- 50-1*diffrhp[[i]]
+ }
> durationphase2WOA<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   durationphase2WOA[[i]] <- ifelse(escalationSAM[[i]]==1,
+                                    (1*slopeWOA*loserrhp[[i]])/2,NA)
+ }
> durationTOTALSAMWOA<- list(length=nrSamples)
> for (i in 1:nrSamples) {
+   durationTOTALSAMWOA[[i]] <- ifelse(escalationSAM[[i]]==1,
+                                      durationphase1SAM[[i]]+
+                                      durationphase2WOA[[i]],
+                                      durationphase1SAM[[i]])
+ }
>
```

5 Statistical analyses

For each assessment strategy, I adjusted linear models between duration and body size in three ways:
1) duration loser body size + winner body size (multiple regression)
2) duration loser body size and duration winner body size (simple regression)
3) duration mean body size (only for size-matched contests)
In this document, I do not show all statistical analysis of this work because the same framework was applied to all assessment strategies. Instead, I only provide an example with pure self-assessment under multiple regression.

5.1 Model creation

```
> modWOA= list(length=nrSamples)
> for (i in 1:nrSamples) {
+   modWOA[[i]] = lm(durationWOA[[i]]~losersize[[i]]+winnersize[[i]])
+ }
```

5.2 Estimation of slopes, standard error of slopes and p-values

```
> loserslopeWOA= list(length=nrSamples)
> for (i in 1:nrSamples) {
+   loserslopeWOA[[i]] = modWOA[[i]]$coefficients[2]
+ }
```

```
> winnerslopeWOA= list(length=nrSamples)
> for (i in 1:nrSamples) {
+   winnerslopeWOA[[i]] = modWOA[[i]]$coefficients[3]
+ }
> SEloserslopeWOA= list(length=nrSamples)
> for (i in 1:nrSamples) {
+   SEloserslopeWOA[[i]] = summary(modWOA[[i]])$coefficients[2,2]
+ }
> SEwinnerslopeWOA= list(length=nrSamples)
> for (i in 1:nrSamples) {
+   SEwinnerslopeWOA[[i]] = summary(modWOA[[i]])$coefficients[3,2]
+ }
> loserpvalWOA= list(length=nrSamples)
> for (i in 1:nrSamples) {
+   loserpvalWOA[[i]] = summary(modWOA[[i]])$coefficients[2,4]
+ }
> winnerpvalWOA= list(length=nrSamples)
> for (i in 1:nrSamples) {
+   winnerpvalWOA[[i]] = summary(modWOA[[i]])$coefficients[3,4]
+ }
```