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ORIGINAL PAPER

Variable assessment of wing colouration in aerial contests of the red-winged damselfly *Mnesarete pudica* (Zygoptera, Calopterygidae)

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Abstract Wing pigmentation is a trait that predicts the outcome of male contests in some damselflies. Thus, it is reasonable to suppose that males would have the ability to assess wing pigmentation and adjust investment in a fight according to the costs that the rival may potentially impose. Males of the damselfly *Mnesarete pudica* exhibit red-coloured wings and complex courtship behaviour and engage in striking male–male fights. In this study, we investigated male assessment behaviour during aerial contests. Theory suggests that the relationship between male resource-holding potential (RHP) and contest duration describes the kind of assessment adopted by males: self-assessment, opponent-only assessment or mutual assessment. A recent theory also suggests that weak and strong males exhibit variations in the assessment strategies adopted. We estimated male RHP through male body size and wing colouration (i.e. pigmentation, wing reflectance spectra and transmission spectra) and studied the relationship between male RHP and contest duration from video-documented behavioural observations of naturally occurring individual contests in the field. The results showed that males with more opaque wings and larger red spots were more likely to win contests. The relationships between RHP and contest durations partly supported the self-assessment and the mutual assessment models. We then experimentally augmented the pigmented area of the wings, in order to evaluate whether strong and weak males assess rivals' RHP through wing pigmentation. Our experimental manipulation, however, clearly demonstrated that strong males assess rivals' wing pigmentation. We finally suggest that there is a variation in the assessment strategy adopted by males.

Keywords Dragonfly · Game theory · Territoriality · Competition · Ornament

Introduction

In many winged insect species, males engage in aerial contests for access to resources, such as mates and oviposition sites (Kemp and Alcock 2003). Since these interactions are energetically costly (Marden and Waage 1990), males are expected to use decision rules to optimize energy expenditure and adjust investment according to the benefits and costs of fights (Enquist and Leimar 1983; Keil and Watson 2010; Hardy and Briffa 2013). The current theoretical framework (Enquist and Leimar 1983; Taylor and Elwood 2003; Gammel and Hardy

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2003; Arnott and Elwood 2009; Bradbury and Vehrencamp 2011; Elwood and Arnott 2012) proposes that male assessment may follow one of three different models: the self-assessment model, the opponent-only assessment model or the mutual assessment model.

The self-assessment model assumes that males compete for territories in wars of attrition (WOA). In this model, male–male interactions usually involve non-contact escalated flying displays ending when one of the contestants reaches an internal physiological threshold (Kemp and Alcock 2003; Marden and Waage 1990). In other words, there is an energetic state threshold, below which, males can no longer sustain a fight and thus withdraw. Alternatively, males may follow an opponent-only assessment model (Arnott and Elwood 2009; Elwood and Arnott 2012), where males are able to assess rivals, but not their own fighting ability. Finally, the mutual assessment model (Elwood and Arnott 2012) has three variations namely, the hawk/dove game theory (Maynard-Smith and Price 1973), which predicts that males may adopt two behavioural strategies: acting as a hawk and always trying to injure the opponent, or acting as a dove and adopting less aggressive behaviours against other doves and always retreating against hawks. The other two variations of the mutual assessment model predict that rivals perform increasingly costly behaviours (sequential assessment model (SAM)) (Enquist and Leimar 1983) or escalate to aggression (cumulative assessment model, CAM) (Payne 1998), to obtain information about their relative strength and to allow assessment of their own winning chances. This behaviour results in the withdrawal of the weakest contestant (Enquist and Leimar 1983; Taylor and Elwood 2003).

According to recent theoretical and empirical analyses, such models are based on the assessment of male resource-holding potential (RHP) (Parker 1974) by contestants (Kemp et al. 2006a; Small et al. 2009; Constant et al. 2011). A myriad of morphological and behavioural traits may act as indicators of male fighting ability such as body size/mass (Kemp et al. 2006a; Guillermo-Ferreira and Del-Claro 2012; Peixoto and Benson 2011), age (Guillermo-Ferreira and Del-Claro 2011), ornament colour/size (Contreras-Garduño et al. 2008; Guillermo-Ferreira and Del-Claro 2011; Kuitunen and Gorb 2011) and flight performance (Kemp et al. 2006b). The three models described above suggest that the relationship between contest duration and the RHP of winners and losers may predict the assessment strategies applied by fighting males.

In the self-assessment model, the prediction is that contest duration increases with loser RHP, but there is no or a minor relationship between winner RHP and duration (Mesterton-Gibbons et al. 1996; Arnott and Elwood 2009; Bradbury and Vehrencamp 2011; Elwood and Arnott 2012), indicating that male decisions are solely based on their own energetic status (self-determined persistence, Taylor and Elwood 2003). In the opponent-only assessment model, winner RHP should increase with contest duration, while no relationship should be found for loser RHP (Elwood and Arnott 2012). Finally, the mutual assessment model predicts that contest duration should decrease with increasing winner RHP and increase with increasing loser fighting ability (Taylor and Elwood 2003; Elwood and Arnott 2012). The mutual assessment model also predicts that contest duration should be longer if rivals are RHP-matched, and thus, duration should be negatively correlated with winner–loser RHP asymmetry, which is not the case for the self-assessment model where there is no such relationship (Bradbury and Vehrencamp 2011).

Nevertheless, recent studies have brought attention to intraspecific variation of assessment strategies (e.g. Hsu et al. 2008; Kasumovic et al. 2011; Palaoro et al. 2014) and to species that exhibit assessment models that were not predicted by theory (Jennings et al. 2005; Stuart-Fox 2006). Thus, Mesterton-Gibbons and Heap (2014) proposed a theoretical model for explaining the concept of variable assessment, based on the hawk/dove game theory (Maynard-Smith and Price 1973). It was assumed that males can gather reliable information on their own RHP, but rival assessment strategy is variable. Hence, assuming that individuals can either be weak or strong, Mesterton-Gibbons and Heap (2014) suggest that there is a difference in payoffs between weak and strong individuals that can significantly affect the benefits of mutual assessment. For instance, in species where contests are costly, weak individuals should avoid contests, but strong individuals should benefit from fighting. Therefore, mutual assessment would not be adopted by weak individuals, but by strong individuals to avoid costly escalation against matched opponents. In other words, variation in assessment strategies is a result of a trade-off between investment in mutual assessment and the capacity to escalate (Mesterton-Gibbons and Heap 2014).

Thus, here, we investigated the assessment model adopted by males of the damselfly *Mnesarete pudica* Hagen in Selys (Zygoptera: Calopterygidae), addressing whether there is a variation of assessment strategies according to male RHP (Mesterton-Gibbons and Heap 2014). By virtue of their complex fighting behaviour, dragonflies and damselflies have been previously used as model organisms in studies investigating aerial contests (Suhonen et al. 2008; Junior and Peixoto 2013). In calopterygid damselflies, wing pigmentation is a condition-dependent ornament that correlates with male fat reserves, immune competence and male quality (Córdoba-Aguilar 2002; Contreras-Garduño et al. 2006; Contreras-Garduño et al. 2007a; Serrano-Meneses et al. 2007). Optical properties of wings (Hooper et al. 1999) and body coloration (Fitzstephens and Getty 2000) also seem to be condition-dependent signals that may influence territorial behaviour (but see Contreras-Garduño et al. 2007b) and rival recognition (Guillermo-Ferreira et al. 2014). These traits are indicators of male RHP and influence the outcome of male–male contests, which are finally related to territory acquisition/defence and mating success (Contreras-Garduño et al. 2008; Guillermo-Ferreira and Del-Claro 2011). Since there is a variation in male RHP and fighting tactics in damselflies (e.g. Raihani et al. 2008; Golab et al. 2013), it is expected that there should also be a variation in the assessment strategies adopted by weak and strong individuals (Mesterton-Gibbons and Heap 2014).

Calopterygid males engage in aerial contests with rivals, fighting for territories and females. These fights may proceed in one of two manners: (i) pursuit fights (<15 s) (Waage 1988), when a territorial male chases the rival away from the territory and (ii) long disputes (up to an hour or more), when two males contest for a longer period, presenting threat displays towards each other or flying in circles in a mutual pursuit (Marden and Waage 1990; Guillermo-Ferreira and Del-Claro 2011; Pajunen 1966; Córdoba-Aguilar and Cordero-Rivera 2005), escalating to grabbing and biting in some cases (Rüppell and Hilfert-Rüppell 2013). Long contests in damselflies are energetically costly, depleting male fat reserves (Marden and Waage 1990; Plaistow and Siva-Jothy 1996; Contreras-Garduño et al. 2006), and, thus, generally considered to be WOA (but see Marden and Rollins 1994), in which males fight until one reaches its internal minimum energetic threshold (Payne and Pagel 1996, 1997). In this context, males engage in noncontact displays of endurance and the decision to withdraw from combat is made when the loser reaches its minimum fat reserve threshold level (Marden and Waage 1990; Taylor and Elwood 2003; Arnott and Elwood 2009; Mesterton-Gibbons et al. 1996). However, Grether (1996) found that males with experimentally increased pigmentation defended territories for longer periods, suggesting that males probably have the ability of assessing the pigmentation of rivals. Therefore, it is expected that males adopt a mutual assessment strategy during aerial contests, assessing their own physiological status and the rival's size or colouration, in order to adjust their investment according to costs and benefits of fighting namely, the immunological and energetic costs of aerial displays (Marden and Waage 1990; Contreras-Garduño et al. 2006) and the benefits of defending a territory (i.e. mating success).

In order to test the theoretical assessment models (Enquist and Leimar 1983; Taylor and Elwood 2003; Gammel and Hardy 2003; Arnott and Elwood 2009; Bradbury and Vehrencamp 2011; Elwood and Arnott 2012) in *M. pudica*, we studied the relationship between contest duration and male RHP by observing individual contests in the field. Male RHP was estimated through body size and measurements of wing pigmentation, reflectance and transmission spectra of individual males, because wing pigmentation (Contreras-Garduño et al. 2006) and optical properties of wing colouration (Schultz and Fincke 2009) can be used as cues for male quality. Thus, we assumed that these variables are potential signals and predictors of male RHP and endurance (Guillermo-Ferreira and Del-Claro 2011; Contreras-Garduño et al. 2008; Grether 1996). We hypothesized that males adopt a mutual assessment strategy, and thus, contest duration should be negatively correlated with winner RHP and winner–loser asymmetry and positively correlated with loser RHP.

Furthermore, to address whether weak and strong males adopt different assessment strategies (Mesterton-Gibbons and Heap 2014), we analyzed male response to males with experimentally manipulated wing pigmentation (e.g. Grether 1996). We expected that strong males (i.e. with higher RHP) should respond with pursuits and aggression towards males with low pigmentation, because weaker males (i.e. with lower RHP) represent a lower risk of injure. Hence, we also expected that strong males should change their behaviour when facing males with experimentally augmented wing pigmentation. Since stronger males represent a higher risk of injury, males should adopt a non-contact strategy (i.e. aerial threat displays, typical of long contests). If these predictions are confirmed, we may suggest that strong males are able to assess the wing pigmentation of opponents. On the other hand, we expected that weaker males should respond with less aggressive behaviour (i.e. short pursuits), independently of the pigmentation of the rival. Altogether, our two approaches may support the hypothesis that males adopt a variable mutual assessment model during aerial contests (Mesterton-Gibbons and Heap 2014).

Material and methods

Fieldwork was conducted at a stream located in Assis, São Paulo State, Brazil (22° 38′ S, 50° 27′ W; altitude 522 m) from July to December 2012. All males used in this study were mid-aged individuals to avoid influence of age in colour measurements and behavioural tests. Teneral and old males usually do not engage in territorial fights and may exhibit different wing optical properties (Guillermo-Ferreira and Del-Claro 2011; Guillermo-Ferreira et al. 2014). Age was estimated using morphological cues on the wings (Guillermo-Ferreira and Del-Claro 2011; Plaistow and Siva-Jothy 1996).

Naturally occurring contests: contest outcome

To investigate if body size and wing colour parameters (i.e. pigmentation, reflectance and transmission) predict the outcome of fights and, thus, are indicators of male RHP, logistic regressions were performed. A factorial code was assigned to winners (1) and losers (2) of each contest, in order to test if body size and colour parameters are associated with the probability of winning a fight.

Naturally occurring contests: assessment model

Behavioural observations and video recordings of male territorial fights were made from 10:00 to 15:00 in sunny days. The study site was an open field, with high exposure to sunlight. Males were captured and marked with white correction fluid on thorax and abdomen. It is known that this marking method does not change male territorial behaviour (Anderson et al. 2011). We observed 20 individual extended fight displays (>20 s) between 40 males (supplementary video), measured contest duration and identified both the winner and loser of each contest. The male that flew away from the territory after the fight was considered the loser. The male that continued perching on the territory was considered the winner. Winners and losers of each contest were collected after the fight and taken to the lab for further analyses.

Male body size was measured with a digital calliper (0.01 mm), from head to abdomen (excluding appendages). Male wing colouration (Fig. 1a) was measured using four different variables: (i) absolute spot size, which is the area of the four wings covered by the red pigment (in mm); (ii) relative spot size, which is the same area divided by the total area of the four wings (in %); (iii) the wing reflectance spectra, from which brightness, hue, and chroma were calculated; and (iv) the wing transmittance spectra from which mean intensity, hue, and chroma were calculated.

Absolute and relative spot size was measured from pictures using Adobe Photoshop software. The spectral measurements on wings were performed with a combined deuterium and tungsten halogen light source (DH-2000-BAL, Ocean Optics Inc, Dunedin, Florida, USA) illuminating the sample normally to its surface. The light source produced light in a broad spectral range from ultraviolet to near infrared (200 to 1100 nm) that was guided through a 200-µm diameter glass fibre, positioned at a distance of 2 mm above the sample. The reflected light was collected by the same fibre and guided to a spectrometer (HR-4000, Ocean Optics Inc, Dunedin, Florida, USA). Spectra were recorded and processed using the software Spectra Suite (Ocean Optics). The transmittance spectra were measured using the same illumination settings. A detecting glass fibre equipped with a collecting lens collected transmitted light from directly beneath the sample and guided it to the spectrometer. The spectra were measured in five standard pigmented areas of the right hind wing of each male, using the mean of these measured areas as the spectrum for each individual.

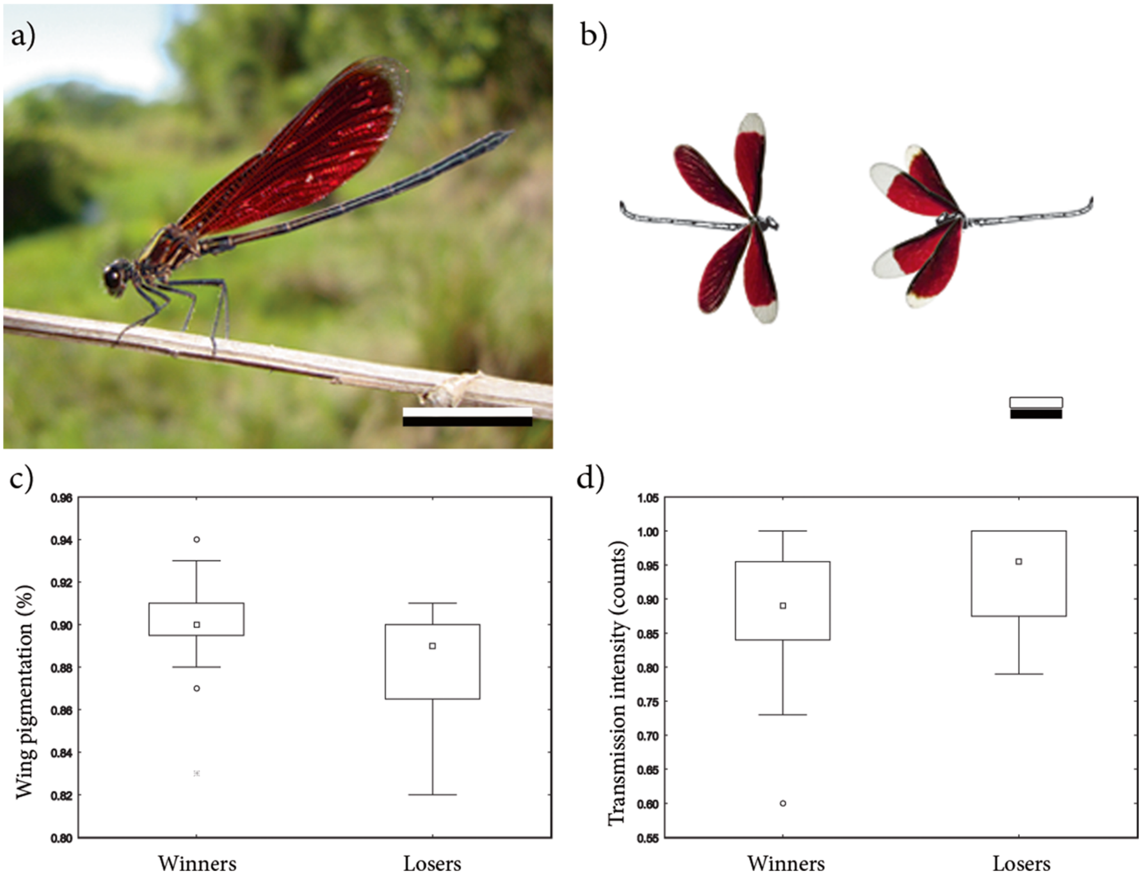

Fig. 1 The wing pigmentation of the male damselfly *Mnesarete pudica*. A male perched on a territory (**a**). The typical threat display and wing pigmentation of winners (*left*) and losers (*right*) (**b**). The probability of winning a fight in relation to male wing pigmentation (**c**). The probability of winning a contest in relation to transmission intensity (**d**). *Scale bars*= 1 cm

To quantify the colour information, we calculated the following parameters: brightness, hue and chroma (Endler 1990), according to the CIELAB colour range. We used the spectra range 300–700 nm, since this is the range of odonate visual receptors (Bybee et al. 2012). Hue was measured as the hue angle of the peak wavelength according to the colour wheel. Chroma was measured as the saturation of the colour and brightness (or intensity) as the integral intensity of light reflected (or transmitted) by the sample. All these measurements were made using the software CLR v. 1.05 (Montgomerie 2008). Similarly, chroma, hue and intensity were determined for transmission.

Simple linear regressions were performed to evaluate the relationship between contest duration and body size and each colour parameter of winners and losers identified as potential indicators of male RHP from the logistic regressions (i.e. relative spot size and transmission intensity, see "Results" section). Furthermore, a simple regression was performed between contest duration and the winner–loser transmission intensity and relative wing pigmentation asymmetries. In the latter, an outlier contest was removed from the analysis. Asymmetry was calculated as the difference between the wing pigmentation (relative spot size) and transmission intensity of winners and losers (i.e. winner RHP – loser RHP). These analyses were made in order to test the self-assessment, opponent assessment and mutual assessment models, based on the predictions described previously.

All statistical tests were run in the Statistica 10 software. Contest duration was log transformed for normalization. Colour variables, body size and wing pigmentation (absolute and relative spot size) were tested for normality through Shapiro–Wilk tests. Values of transmission hue and reflectance brightness were then square root (√) transformed for normalization.

Experimental manipulation: rival assessment

To experimentally investigate if strong and weak males adopt different strategies when assessing the wing pigmentation of rivals, we collected eight males with wing pigmentation lower than 90 % (mean wing pigmentation is equal to 89 %, $N=40$) for further use in a behavioural experiment in the field (tethered males). These males were previously identified as territorial and were individually tethered to a cotton line with a drop of Duco Cement® in the dorsal part of the thorax. The line was then glued to a wood stick, and individuals were presented to other territorial males (focal males) perched on the vegetation on the borders of the stream (e.g. Guillermo-Ferreira et al. 2014). Focal males were divided into weak (pigmentation lower than 90 %) and strong males (pigmentation higher than 90 %). All males were marked with white correction fluid on thorax and abdomen. Male territoriality was assessed through focal animal observations for 10 min, noting if each male was site attached and defended the territory against intruders.

Before the behavioural tests, all eight tethered males had the red area of the wings painted with a red marker (Faber Castell) over the red spot as a sham control. This marker is spectrally similar to the natural pigment colour (Guillermo-Ferreira et al. 2014). First, the tethered males were presented to five territorial males each, totalling 40 focal males.

After these presentations, the four wings of the tethered males were completely painted with the red marker, simulating a male with maximum RHP, and presented to the same territorial males again. This way, there was no variation in body size or body coloration of the tethered males (since the same male was used before and after manipulation), controlling for possible influence of these traits on the experiment. By presenting the tethered males to focal males before and after manipulation, we were able to answer if males change their behaviour according to the rival's pigmentation only.

The behavioural responses of the focal males were recorded and classified as binary data: 1, pursuit, when the focal male rushed towards the tethered male and retreated, without making any threat display, and 2, threat display (Fig. 1b, when the focal male approached the tethered male, hovered exhibiting threat displays and retreated only after the removal of the tethered male (after 20 s)). McNemar change tests were performed in the software SPSS 20® to address whether focal males changed their behaviour after manipulation of the wing pigmentation of tethered males. The sham control was set as factor 1, and the manipulation of wing pigmentation to fully cover the wings was set as factor 2.

Ethical note

The manipulation and collection of the damselflies were conducted under the permanent license numbered 11364-1 from Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), which regulates the protection of wildlife in Brazil. *M. pudica* is not under protection or endangered.

Results

Naturally occurring contests: contest outcome

The first step was to evaluate, among the studied traits, which ones predict the outcome of fights. We consider the variables that predict the outcome of fights as potential representative of male RHP. The probability of winning a fight was predicted by the relative size of the pigmented area (simple logistic regression, $b=32.144$, Wald=4.303, $p=0.038$; Fig. 1c) and transmission intensity (simple logistic regression, $b=-9.128$, Wald=4.844, $p=0.028$; Fig. 1d). The probability of winning a fight was not related to body size (simple logistic regression,

b=0.699, Wald=3.510, p=0.061; Fig. 1d), absolute size of the pigmented area (simple logistic regression, b=1.618, Wald=1.69, p=0.194), transmission chroma (simple logistic regression, b=−14.62, Wald=2.821, p=0.093) and hue (simple logistic regression, b=4.026, Wald=0.831, p=0.362) and reflectance brightness (simple logistic regression, b=1.311, Wald=0.022, p=0.882), chroma (simple logistic regression, b=46.371, Wald=0.779, p=0.377) and hue (simple logistic regression, b=−0.28, Wald=0.054, p=0.815). The second step was to evaluate if the variables considered as predictive of male RHP (i.e. relative spot size and transmission intensity) are assessed by males during contests.

Naturally occurring contests: assessment model

The results indicate that there was a positive relationship between contest duration and loser relative wing pigmentation (simple regression, b=0.521, t=2.591 p=0.018, N=20; Fig. 2a). However, there was no relationship between contest duration and winner relative spot size (simple regression, b=0.044, t=0.189, p=0.853, N=20; Fig. 2b). Loser transmission intensity (simple regression, b=0.16, t=0.66, p=0.948, N=20; Fig. 2c) and winner transmission intensity (simple regression, b=−1.197, t=−0.851, p=0.406, N=20; Fig. 2d) also presented no relationship with contest duration. These results suggest that males follow a self-assessment model, refuting our initial hypothesis that males follow a mutual assessment model. Thus, in this case, wing pigmentation (relative spot size) is probably a trait that is not assessed during fights but may correlate with male fat reserves and persistence in the fight.

There was a negative relationship between fight duration and winner–loser wing pigmentation asymmetry (simple regression, b=−0.577, t=−2.914, p=0.01, N=19; Fig. 2e). There was no relationship between contest duration and transmission intensity winner–loser asymmetry (simple regression, b=−1.76, t=−0.758, p=0.458, N=20; Fig. 2f)

Experimental manipulation: rival assessment

The results show that strong males changed their behaviour, exhibiting more threat displays towards males with augmented wing pigmentation (McNemar test, p=0.001, Fig. 3). Weak males did not change their behaviour, approaching and retreating when facing rivals (McNemar test, p=1.0, Fig. 3).

Discussion

Naturally occurring contests: contest outcome

Herein, we show that aerial contests in the damselfly *M. pudica* are influenced by two visual components of wing pigmentation (relative spot size and transmission intensity). First, the results presented here suggest that the amount of pigment in the wings of males *M. pudica* may be a potential indicator of male RHP during aerial contests. Males with larger areas of the wings covered by the pigment won more fights. This result is similar to other studies on other odonate species (Guillermo-Ferreira and Del-Claro 2011; Contreras-Garduño et al. 2008; Grether 1996). Second, our results also clearly demonstrated that males with lower transmission, and thus higher concentration of pigment (Stavenga et al. 2012), had a higher probability of winning a fight.

Therefore, our results suggest that transmission may be a more important trait than reflectance in territorial fights of calopterygid species with red translucent wings. For instance, transmission is a condition-dependent trait indicating male RHP in the calopterygid *Mnais costalis* (Hooper et al. 1999) and, thus, may be used by males for assessment during fights. Interestingly, a previous study on another red-winged calopterygid damselfly, *Hetaerina americana* (Contreras-Garduño et al. 2007b), has also demonstrated that wing reflectance does not indicate male condition and, therefore, may not play a role in influencing contest outcome and rival assessment.

The fact that transmission influences on contest outcome may explain why most Hetaerininae exhibits red translucent wings instead of the black-pigmented wings of other calopterygids, like *Calopteryx*. *Mnesarete* and *Hetaerina* are tropical genera that inhabit streams and rivers in open areas with high sunlight incidence, what may favour less absorptive wings due to thermoregulation. Since aerial displays must be conspicuous in contrast with the background, translucent wings could also allow males to be conspicuous against the sky during contests and courtship.

Naturally occurring contests: assessment model

According to current theoretical models of male assessment during territorial fights (Enquist and Leimar 1983; Taylor and Elwood 2003; Gammel and Hardy 2003; Arnott and Elwood 2009; Bradbury and Vehrencamp 2011; Elwood and Arnott 2012), our results suggest that males of the damselfly *M. pudica* follow the self-assessment model, because there was a positive relationship between loser's RHP (wing relative spot size) and contest duration, but no relationship between winner's RHP and contest duration. There was also no relationship between fight duration and wing transmission of winners and losers.

These results refute our initial hypothesis, suggesting that males do not follow a mutual assessment model, where fight duration should be negatively correlated with winner's RHP (Elwood and Arnott 2012). Furthermore, males also do not follow the opponent-only assessment model because this model predicts that loser's RHP should not be correlated with

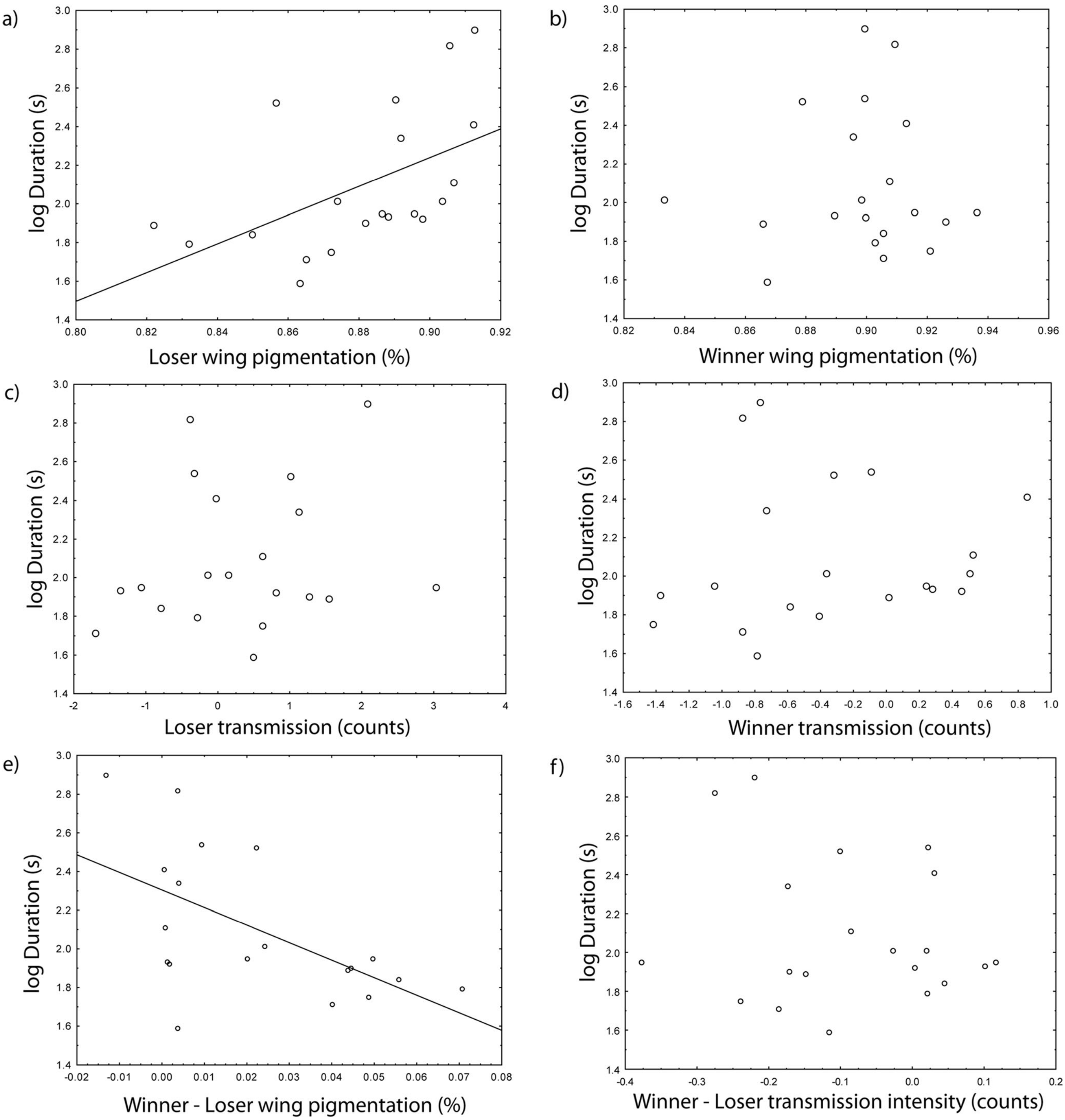

Fig. 2 Relationships of contest duration as a function of winner and loser resource-holding potential (i.e. wing pigmentation and transmission intensity) of males of the damselfly *Mnesarete pudica* (Odonata: Calopterygidae). Relationship between contest duration and wing pigmentation of losers (**a**) and winners (**b**). Relationship between contest duration and wing transmission intensity of losers (**c**) and winners (**d**). Relationship between contest duration and winner–loser pigmentation asymmetry (**e**). Relationship between contest duration and winner–loser transmission intensity asymmetry (**f**). The *lines* indicate significant relationships

contest duration (Elwood and Arnott 2012). Nevertheless, our results support the hypothesis that calopterygid damselflies fight in WOA, contesting until one of the rivals reaches certain physiological threshold (Marden and Waage 1990). The self-assessment model suggests that fight duration should increase with loser's physiological status (Elwood and Arnott 2012). Because male wing pigmentation correlates with fat reserves (Contreras-Garduño et al. 2006), we assume that this trait was a good predictor of male endurance. Nevertheless, there was a negative relationship between contest duration and winner–loser RHP asymmetries, suggesting that fights are longer when rivals are RHP-matched and that males may adopt a

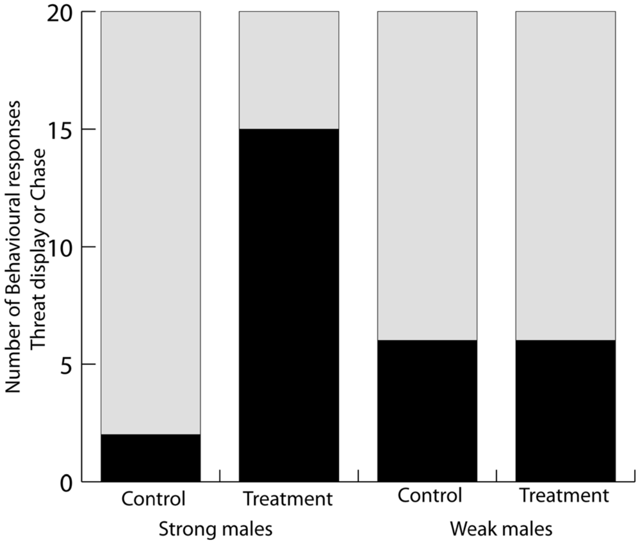

Fig. 3 Results of the experimental manipulation showing the number of threat displays (*black*) and pursuits (*grey*) in male responses towards a sham control (control) and males with wings fully painted (treatment). Strong males changed their response and increased the number of threat displays when pigmentation of the rival was augmented, but weak males did not

mutual assessment strategy (Bradbury and Vehrencamp 2011), which supports our initial hypothesis. In any case, males may assess their own internal physiological status when deciding to withdraw from combat and ultimately assess the pigmentation of the rival.

Experimental manipulation: rival assessment

The behavioural experiment corroborates our initial hypothesis because strong males changed their behaviour according to the wing pigmentation of the rival, but weaker males did not. Strong males responded with pursuits and physical attacks towards males with smaller wing spots but exhibited threat displays against males with experimentally augmented wing pigmentation. These results suggest that males were able to assess the wing pigmentation of opponents and adjust investment in fighting according to the costs that the rivals impose. Stronger males may represent a higher injury risk and thus, be more cautiously approached by other males (i.e. with noncontact threat displays), which are the typical behaviour of long and energetically costly contests. As proposed in Mesterton-Gibbons and Heap (2014), because contests are costly in *M. pudica*, weak individuals exhibit reduced investment towards assessment and avoid long contests, but strong individuals benefit from such fights and adopt a mutual assessment strategy to avoid wasting energy with weaker opponents and costly escalation against matched opponents.

In this scenario, the variation in male assessment is a result of the trade-off between the costs of assessment and the capacity to escalate (Mesterton-Gibbons and Heap 2014). In other words, strong males first assess the RHP of the rival (mutual assessment) and choose to switch to a self-assessment-based WOA (i.e. noncontact long threat displays) if the opponent may impose higher costs (e.g. injury). Alternatively, long contests in calopterygids usually involve several aerial displays, like flying in circles, rocking flights and lateral and frontal hovering displays (Pajunen 1966), which may be a strategy adopted by males that fit the SAM, in which males engage in sequences of complex behavioural displays until the rival is not able to perform them (Enquist and Leimar 1983). Weak males have lower fat content, and thus less energy to fight (Contreras-Garduño et al. 2006), have less juvenile hormone and thus are less aggressive and exhibit lower escalation capacity (Contreras-Garduño et al. 2009).

When we tested the theoretical predictions of the assessment models (Taylor and Elwood 2003; Gammel and Hardy 2003; Elwood and Arnott 2012), our results fitted the self-assessment model and partly the mutual assessment model (Elwood and Arnott 2012). Although these results corroborate previous findings (Marden and Waage 1990), the conflict resolution theories that we tested here in this study (Bradbury and Vehrencamp 2011; Elwood and Arnott 2012) were probably not sensitive to the assessment behaviour of *M. pudica* males. By manipulating male wing pigmentation, we showed that strong males are able to assess the wing pigmentation of opponents (i.e. mutual assessment model), while weak males probably adopt a self-assessment model. The combination of both approaches in this study corroborates our initial hypothesis and the theory proposed in Mesterton-Gibbons and Heap (2014) that males of *M. pudica* exhibit variations in assessment strategies adopted during aerial contests.

Recent discussion, devoted to the explanation of assessment of fighting abilities in animals, has emphasized the importance of considering that the assessment of fighting ability need not be cognitively complex, and thus, simple explanations are enough for modelling assessment (Fawcett and Mowles 2013; Elwood and Arnott 2013). However, based on our results, we suggest that even animals with simple nervous systems (e.g. arthropods) may exhibit complex assessment strategies. For instance, males of the decapod *Aegla longirostri* adopt a mixed assessment strategy, based on both chemical clues and disputes by claw grabbing (Palaoro et al. 2014). Here, *M. pudica* males may also adopt a mixed strategy. We found that wing colouration (i.e. spot size and colour opacity) predicted the result of disputes. Although fight duration had no relationship with wing transmission, this trait may directly affect the conspicuousness of the multicomponent signal that is assessed by males during aerial contests. For instance, wing transmission is an indicator of the amount of pigment in the cuticle, which may enhance the visibility of the size of the red spot in open field and sunny areas.

Conclusion

Considering the complexity of signals from multiple colour parameters (e.g. Guillermo-Ferreira et al. 2014), the complex behavioural displays (Guillermo-Ferrera and Bispo 2012) and the cognitive ability to interpret them, we conclude that the assessment strategies adopted by *M. pudica* males may not be explained by a single model. Indeed, Mesterton-Gibbons and Heap (2014) proposed a theoretical treatment in which the type of assessment depends on the intensity of the contest, explaining variations in assessment strategies.

Therefore, we suggest that strong males probably adopt a mixed two-step decision rule: first, based on the pigmentation of the rival, males decide to engage in a long contest or to fight briefly with aggression and pursuits; second, after engaging in a long fight, males fight in WOA or follow the SAM. The WOA proposed by Marden and Waage (1990) may only occur when the opponent is a strong male (i.e. large wing spotted), when threat displays, typical of longer fights, can be observed. In this context, males engage in aerial threat displays until one reaches its physiological threshold and withdraw. Alternatively, males may follow a SAM and engage in aerial displays of increasing difficulty (Pajunen 1966), until one is not able to perform a display and give up. Against weaker opponents, males tend to solve the conflict by using less energetically costly and more aggressive behaviours such as chasing, grabbing and biting. Weaker males, which have less energy to fight, adopt a less costly strategy and avoid the costs of assessment by engaging in brief pursuits without aerial displays.

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