

# The size of the red wing spot of the American rubyspot as a heightened condition-dependent ornament

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We investigated an ornamental trait known to reflect male fighting ability and tested whether it shows heightened condition dependence compared with nonornamental traits in the American rubyspot (*Hetaerina americana*). Adult males bear red wing spots, the size of which is sexually selected: large-spotted and fatter males are more successful in territorial competition and obtain more matings than are nonterritorial males. First, to see whether spot area may signal fighting ability at a particular age (to discriminate animals that are unlikely to compete), we investigated the age at which males engaged more in fighting and compared their fat reserves and muscle mass at 3 ages (young, middle aged, and old) and territorial status. Middle-aged males showed the highest fat and muscle values, engaged more in fighting, and were predominantly territorial. Second, we looked for traits not shaped by sexual selection: we compared red chroma and brightness of spot and thorax, spot area, muscle mass, and fat reserves in winner and loser males after a territorial contest. The only difference was that winners had larger spot areas and higher fat reserves. Finally, an immune challenge-based experiment was performed during the development of spot area and its color properties (chroma and brightness). Compared with a control (unchallenged) group, the results revealed that area decreased, brightness increased, and there was no change in red chroma, muscle mass, and fat reserves in challenged animals. Thus, spot area is a stress-sensitive, energy-reflecting trait that is likely to be used for communication during territorial competition in these damselflies. *Key words*: American rubyspot, energetic signaling, heightened condition dependence. [*Behav Ecol* 19:724–732 (2008)]

One key question in sexual selection theory is what information individual males convey to conspecifics through their ornaments. Related to this, there is extensive evidence supporting the notion that in many cases, ornament expression correlates with male condition (or “quality”; reviewed in Andersson 1994; Johnstone 1995). Condition is a general term, which is interpreted as a trait closely linked to viability (Iwasa et al. 1991; Iwasa and Pomiankowski 1994). It is thus assumed that there exists a positive relationship between condition and an individual’s fitness. Condition can be also seen as the amount of resources available for allocation to fitness-enhancing traits (Lorch et al. 2003; Tomkins et al. 2004), given the assumption that all these traits are costly to produce, which thus can result in production and maintenance conflicts if the individual has not accrued enough resources. The positive relationship between ornament expression and condition has been thought to be the way by which individuals honestly communicate their condition (Zahavi 1975). The honesty of such condition-reflecting ornaments prevents those males in a poor condition from cheating (i.e., being unable to produce and/or maintain costly ornaments) (Zahavi 1975; Andersson 1986).

An accepted idea regarding the production and/or maintenance of ornaments is that they are condition dependent (reviewed by Andersson 1994; Johnstone 1995). One basic assumption of this idea is that, for example, only those males

that are able to gather sufficient dietary resources will be able to produce and maintain the most conspicuous ornaments. However, such relationships should be interpreted carefully because the majority of traits are expected to show a degree of condition dependence. In fact, it is expected that ornaments, unlike nonornamental traits, must reflect heightened condition-dependent expression (Grafen 1990). One recent case, for example, provided evidence that structural-based wing color was more indicative of developmental stressors (via diet and sudden changes in temperature) than pigment-based colors in male butterflies (Kemp and Rutowski 2007). Detailed cases like this are scarce in the literature, which led Cotton et al. (2004b) to propose that most experimental evidence gathered so far derives from studies that lack comparisons of condition dependence and expression of both ornamental and nonornamental traits, thus failing to show heightened condition dependence for the former traits.

In this study, we investigated whether male fighting ability in a calopterygid damselfly is signaled during territorial competition and whether the ornamental trait that serves this signaling function has a heightened condition-dependent nature compared with nonornamental traits. We used the American rubyspot (*Hetaerina americana*) as our model organism. Males of this species defend riverine territories consisting of a few perching sites where females visit to mate (Grether 1996a). These territories do not bear any material benefit for the female because once the couple is formed, females oviposit their eggs at an alternative site, which contains the submerged plants that females use for inserting their eggs (Raihani et al. forthcoming). The nature of male aggregation in this species led Raihani et al. (forthcoming) to suggest that animals in this

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species practice a lek mating system. Territorial defense takes place by using a series of ascending and descending flights of circular trajectory in which both males repeatedly face each other (Johnson 1962). When a female arrives to a territory, in the absence of any precopulatory behavior, the territorial male grabs her and, invariably, mates with her (Bick and Sulzbach 1966). Similar to other calopterygids (reviewed by Córdoba-Aguilar and Cordero-Rivera 2005), American rubyspot territorial residents accrue a significantly higher mating success compared with nonterritorial males (i.e., individuals that were not able to acquire a territory; Grether 1996a; Serrano-Meneses et al. 2007). Mating success seems reliant mainly on male–male competition, rather than female choice, at least during the precopulatory stage (Grether 1996a). The probability of fighting for and defending a territory in this and other calopterygids is dependent on energetic differences between territorial and nonterritorial males: individuals with more fat are more likely to win a territorial dispute (for the American rubyspot, see Serrano-Meneses et al. 2007; for other species, see Marden and Waage 1990; Plaistow and Siva-Jothy 1996; Kōskimäki et al. 2004; Córdoba-Aguilar et al. 2007). Males unable to win these fights remain nonterritorial throughout their life span. There is even a third male mating tactic, termed switchers, in which males switch from periods of territoriality to nonterritoriality and back to territoriality again (Raihani et al. forthcoming). But still within this last tactic, the switchers, fatter males, are more successful in obtaining a territory after a period of nonterritoriality (Raihani et al. forthcoming). Territorial success is also correlated with patterns of wing pigmentation in members of this family. For instance, in the case of the American rubyspot, recent evidence has shown that males with larger wing pigmentation areas are more likely to obtain and/or defend a territory (Grether 1996a, 1996b; Contreras-Garduño et al. 2006; Serrano-Meneses et al. 2007; Raihani et al. forthcoming).

Soon after emergence, but prior to sexual maturity (the “teneral” stage, characterized by an absence of fixed color except for the wing spot; Contreras-Garduño J, unpublished data), the American rubyspot male spends from 2 to 7 days foraging for food (Grether 1996b). It then takes approximately 20 days for the young male to complete the wing pigmentation area and color as well as thoracic muscle mass and fat reserves, which serve to fuel flight (Contreras-Garduño J, unpublished data; for data on a closely related species, see Córdoba-Aguilar 1993). The pigmentation area in this species appears as 4 spots, each one located at the base of each wing, covering approximately 15–27% of wing area (Grether 1996b; Contreras-Garduño et al. 2006). Once the spots are fully developed and fixed, the now middle-aged male becomes sexually mature and active (Grether 1996b), a period that lasts from 1 to 2 or 3 weeks (Contreras-Garduño J, unpublished data). It is also at this time that other body parts, such as the thorax, have developed a strikingly conspicuous coloration (for another *Hetaerina* species, see Córdoba-Aguilar 1993). There is a positive correlation between spot area, fat reserves, and muscular mass in those middle-aged males that compete for territories (Contreras-Garduño et al. 2006; Serrano-Meneses et al. 2007). This relationship has led to the hypothesis that the wing spot area functions to signal male energetic condition during contests at an age when such competition takes place (Contreras-Garduño et al. 2006). The observed relationship between spot size and fat may be explained if only those males that have accrued enough resources during the larval and teneral stages are able to produce large spots and obtain high fat reserves stored when sexually mature (for data suggesting this in other species, see Hooper et al. 1999; Plaistow and Siva-Jothy 1999; Plaistow and Tsubaki 2000). Nevertheless, spot area may not be the only

variable shown during contests, given the colorful and conspicuous nature of both the spot and the thorax of these animals. In fact, one recent study in another calopterygid, *Calopteryx maculata*, has shown that thoracic color correlates with energetic condition in fighting males: young, blue males were more likely to have larger fat reserves than old, green individuals (Fitzstephens and Getty 2000). In that study, color was assumed to indicate male energetic condition, as experimental manipulation of diet quantity affected the rate of color change over time. Although we found in a previous study that color properties (chroma and brightness) of both the spot and the thorax did not reflect territorial status, age, or condition (when diet or immune stressed; Contreras-Garduño, Buzatto, et al. 2007) in American rubyspot males, this was not assessed at the time when fighting takes place which is when signaling of energetic condition should occur. After displacement from a territory, the old male wanders through territories as a nonterritorial male, although these males may occasionally participate in territorial contests (Serrano-Meneses et al. 2007). However, how frequently these different-aged males engage in territorial disputes has not been investigated. This information is important because fat levels do not keep constant with age (Plaistow and Siva-Jothy 1996). Therefore, given the assumption that ornaments signal energetic levels and thus likely fighting ability, any communication should occur at an age when fat and muscle are at their highest value.

We first determined the ages of those males that were involved in territorial contests to see whether males differed in their time spent fighting at different ages. This is key because although males of different ages may show similar variation in ornament expression (e.g., spot size), if males of a particular age are more likely to be involved in territorial contests, selection will operate on this age. We also compared fat load, muscular mass, and territorial status of males of different ages. We predict that because middle-aged males show the greatest fat (Contreras-Garduño et al. 2006) and muscle (Serrano-Meneses et al. 2007) value variation, these males should spend more time fighting over territories and will be more likely to act as territorials. Second, we examined differences in wing spot area and color properties of this trait and the thorax in both winners and losers of a territorial contest to determine the traits that may be used as a signaling ornament. The specific traits we measured were spot area and red chroma and brightness of both the spot and the thorax. We selected these color properties based on color perception findings in other animals, which have been shown to ultimately affect behavioral decisions (e.g., mammals, Krupa and Geluso 2000; birds, Saks et al. 2003; reptiles, Whitting et al. 2006; fish, Marshall et al. 2003; insects, Katsoyannos et al. 1985). Once we compared these traits, differences in fat reserves and muscular mass were also compared to provide a physiological measure of likely fighting ability. These comparisons were carried out while controlling for body size for the following reasons (see Cotton et al. 2004b): 1) the need to remove the effect of this trait given its likely allometric relationship with the ornamental or nonornamental trait; 2) to eliminate the probability that it is body size, and not the ornamental trait, that is being sexually selected (in this case, body size being assessed by competitors); and 3) to see to what extent the ornament entirely reflects condition outside its allometric relationship with body size. Third, to know if a potential competitive ability signal (i.e., the variables that we found to be related with the contest outcome) is more sensitive to male condition than a nonornamental trait (i.e., the variables that we found to have no relationship with being a winner or a loser in a contest), we performed an experiment in which recently emerged teneral males were immune challenged with a nylon monofilament

(using the rationale that the immune response entails a considerable energetic cost for the individual; Rantala et al. 2000; Siva-Jothy 2000; Kösikmäki et al. 2004; Contreras-Garduño et al. 2006). Using an immune protocol that truly simulates a response to a pathogen attack (Rantala and Roff 2007), the goal of this experiment was to induce the animal to face a resource allocation conflict between both trait types at an age when resources are being accumulated and are at stake. We predicted that the ornamental traits in experimental males would be more affected compared with nonornamental traits.

## MATERIALS AND METHODS

### Study site, ages of contesting males, fat load, and muscular mass at different ages and status

Fieldwork was carried out in the Xochitepec river (18°32'56"N, 99° 16' 23"W), Morelos, Mexico in April and May 2007. We collected 2 sets of males. The first set comprised males that were involved in true contests. These males were collected to determine their age. We used 3 age categories similar to our previous works (e.g., Serrano-Meneses et al. 2007): 1) juvenile mature, shiny-winged males in which the development of body color is incomplete; 2) middle-aged mature animals with bright body color and highly transparent wings; and 3) old mature animals with dark or pale coloration, abundant pruinosity, and fragile wings that are less transparent. The second set comprised males of different ages to determine their fat reserves, muscular mass (see below for a description of fat and muscular mass determination), and territorial status (territorial and nonterritorial). A territorial male typically chases away other conspecific males when they come within 1 m of his territory and returns to his original perch once the intruder has been diverted. Territorial males remain in the same territory for at least 1 day. Males that did not display this type of behavior were termed nonterritorial (for a similar rationale, see Córdoba-Aguilar and Cordero-Rivera 2005).

### Contest outcome

Observations and male collection were carried out in the same place during May 2005. We observed males that engaged in "true" territorial contests ( $N = 14$ ), and the identity of winners and losers was recorded after each event. We considered "true contests" to be aggressive interactions in which males exhibited ascending and descending flying trajectories that lasted more than 2 min. These contests are, in fact, more likely to result in a change of territorial roles compared with shorter encounters (Contreras-Garduño et al. 2006). Males that remained in the disputed area were considered winners, whereas those that fled several meters away from the site were regarded as losers. After each contest, we netted both males to measure spot area, spot color (red chroma and brightness), thoracic color (red chroma and brightness, see below for a description of spot size and color measurement), thoracic fat, thoracic muscle mass, and forewing length.

### Condition dependence experiment

During July 2005, last instar larvae were collected from the same river and taken to the laboratory until emergence. The larvae were individually allocated (to avoid any conspecific aggression) and fed with chironomid larvae ad libitum.

We randomly designated newly emerged teneral adults (after 1 h of emergence) to 1 of 2 groups that differed in

immune challenge intensity. In the experimental group ( $N = 8$ ), we subjected males to an immune challenge consisting of a previously disinfected (with 90% ethanol) nylon monofilament (1 mm length, 0.2 mm diameter) that was inserted through the 4 abdominal pleura on the ventral midline using fine forceps. The monofilament was replaced by a new one every 4 h, giving a total of 6 monofilaments during 24 h (the total duration of the experiment). This regular immune cost on the animal should promote a continuous resource allocation to eliminate the "pathogen" (for a similar methodology and nature of the assumed cost, see Rantala and Roff 2007). In the control group ( $N = 8$ ), we handled males in the same way as experimental ones, but the monofilaments were not inserted. Twenty-four hours after the manipulation, we also measured spot area, spot red chroma, spot brightness, muscle mass, fat reserves, and body size (as head width; see below for color and head width measurement). Unlike our measurement of body size in fighting males as forewing length, in this experiment, size was measured as head width because wings are extremely difficult to handle and manipulate in the teneral stage and both wing length and head width are good indicators of body size (Serrano-Meneses et al. 2007). The latter was tested by measuring both traits in 20 males that were collected in the same period. The correlation between both traits was highly significant ( $r_{\text{pearson}} = 0.854$ ,  $P < 0.0001$ ).

Ideally, many more measures of potential nonornamental traits would have made our experiment more complete. However, the options we had were not either viable or convenient. For example, we did not include the chroma and brightness of thorax because color is not yet developed in adults that are 1-day old (Contreras-Garduño J, unpublished data). Furthermore, we only used one measure of body size (head width) as the other commonly used measures (wing and abdominal lengths) were not only manually difficult to measure (most tissues are extremely soft and fragile so that measurements can be highly inaccurate) but also highly correlated with each other (Serrano-Meneses et al. 2007). Furthermore, it may be also argued that spot color traits may not be independent from spot size, and therefore, comparing all these traits may not be biologically coherent. This is why we used 2 more traits, muscle mass and fat reserves, which should be independent from the spot.

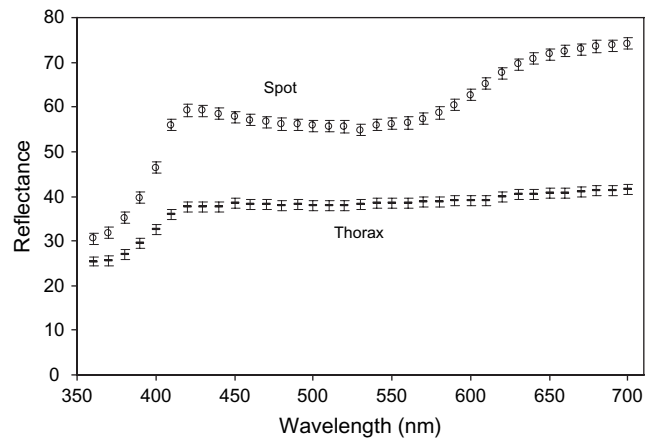
### Spot area and spot and thoracic color

To measure spot area, we cut the right forewing of each male (from its insertion to the thorax) and took a digital photograph of the entire wing (Canon digital camera, Power Shot G6) in the laboratory using artificial light conditions. From these photographs, we calculated the relative percentage of pigmented areas using Image Tool for Windows® version 3.0 (see similar procedure in Contreras-Garduño et al. 2006). These areas appear clearly defined in the computer screen so there is no need for specific photographic conditions (such as the use of filters). The observer was always "blind" to the sample of origin. Regarding color, we recorded the chroma and brightness values for the spot (right anterior wing) and thorax. For this, we used a hand-held, sphere-type spectrophotometer (Minolta CM-2500C), which is appropriate for small and irregular surfaces. This device uses the Spectra Magic NX Color Data software (for more details, see <http://konicaminolta.com/instruments/products/color/spectrophotometer/cm2500c/index.html>). This apparatus has a wavelength range of 360–740 nm (at 10-nm intervals), a reflectance range of 0–175%, and a resolution of 0.01%. It contains a mixing box (based on an integrating hemisphere) and works by making complete contact with the sample whose

color is to be recorded (which avoids the entrance of any external light source that may affect color recording). It bears an illumination slit created by a ring-shaped light source (via a 2 pulsed xenon lamps), which is received back by a silicon photodiode array (dual 40 elements). The reflectance spectra of each of the measurements are obtained as the mean of 3 sequential measurements that the spectrophotometer automatically records. Each measurement takes approximately 1.5 s while the interval between measurements is 4 s. Color measurements that this device is based on are defined according to the CIELab system (Anderson and Prager 2006; Quesada and Senar 2006). Chroma and brightness can be recorded using this device (e.g., Torres and Velando 2003; Contreras-Garduño, Buzatto, et al. 2007).

Some problems arising when assessing color instruments based on human vision (for an extended view of these problems, see Endler 1990) can be solved by using spectrophotometers based on CIELab measurements (Anderson and Prager 2006). More specifically, some of the main problems are caused by ambient light (*A*), reflectance (*R*), transmittance (*T*), and receiver sensitivity (*S*) (Endler 1990; Anderson and Prager 2006). According to our methodology, *A* was controlled as the spectrophotometer makes direct contact with the body region impeding external light sources to interfere with the recording; *R* and *T* are measurements given in the reflectance obtained by the spectrophotometer (Anderson and Prager 2006) while *S* (red chroma) was included within the range that odonates are able to see (from less than 600 up to 700 nm; Chapman 1998; Briscoe and Chittka 2001).

Odonates can potentially see within a wavelength ranging between 330 and 630 nm (e.g., *Hemicordulia tau*, Yang and Osorio 1991; *Sympetrum rubicundulum*, Meinertzhagen et al. 1983), which is one of the largest ranges in the animal kingdom (300–700 nm; Briscoe and Chittka 2001). This ability is based on 2 unique particularities within insects and, probably, among animals in general (Briscoe and Chittka 2001): 1) the large number and types of spectral receptors (up to 6 different types, e.g., *S. rubicundulum*; Meinertzhagen et al. 1983), which code for particular wavelengths and are located in distinct eye regions, and 2) different chromophore-based photopigments (types A1 and A3, unlike many other insects which have only one type; Seki and Vogt 1998). The photopigments are located within the receptors, and the ability to discriminate color will depend on which wavelength enters particular regions of the eye (which in many cases serve as a filter) before reaching the photopigments (Chapman 1998). However, these 2 particularities mentioned above allow odonates to potentially discriminate between UV, blue, green, and red patterns (Meinertzhagen et al. 1983; Labhart and Nilsson 1995; Yang and Osorio 1996). Nevertheless, the extent to which the neural properties of odonates allow them to distinguish between such colors and take a decision is an open question (as it is in insects in general, Briscoe and Chittka 2001). The color vision ability is thus inferred from what the structure in question reflects. For example, Fincke et al. (2007) recently documented the color reflectance peak of the thorax in *Enallagma* damselflies, which was between 350 and 475 nm and thus inferred animals were able to detect UV patterns. Of course, such an approach is preferable to previous measurements where human-based assessment of color was a standard methodology (e.g., Gorb 1998). Following such rationale and practice, for measuring chroma and brightness, we first determined the reflectance of both the spot and the thorax by using 40 males (20 territorial and 20 nonterritorial males) collected in the same site and in July 2005. Given the wavelength, the peak was between 600 and 700, whereas the total reflectance range is found between 360 and 740, and (Figure 1) we used



**Figure 1**  
Mean reflectance spectra of the wing spot and thorax of 40 males (20 territorial and 20 nonterritorial). Bars are standard deviation.

the former for measuring red chroma (as red reflectance/damselfly's total reflectance [ $R_{600-700}/R_{360-740}$ ] as suggested by Montgomerie 2006) and the latter for brightness (as total reflectance,  $R_{360-740}$ ).

We directed the light source of the spectrophotometer by making direct contact with the body region (the “capture area” was the central area of the spot and thorax and a circular surface of approximately 0.2 cm of radius; this capture area is large enough to cover a reasonable “sample” of the structure as the approximate dimensions are of 0.96 cm in length by 0.48 cm in width for the spot and 0.76 cm in length and 0.47 cm in width for the thorax). Color recording was done at midday (1100–1500 h—the time at which animals are active in territories) inside a naturally illuminated room. Calibrations with respect to zero and white standards were performed according to the manufacturer’s instructions and each time the device was turned on (see Quesada and Senar 2006). To see how consistent measurements were, we compared the reflectance of 2 different measures of the spot, taken within 10 min of difference between each record for the same 40 males used for detecting the peak reflectance described before. These measures were highly repeatable for chroma ( $r_{\text{pearson}} = 0.94$ ,  $P < 0.0001$ ) and brightness ( $r_{\text{pearson}} = 0.96$ ,  $P < 0.0001$ ).

**Muscle mass, fat load, and body size**

Muscle mass was measured by immersing the thorax in potassium hydroxide (0.2 M) for 48 h (Plaistow and Siva-Jothy 1996). The weight of this body region was measured previous to and after the extraction, and the difference was interpreted as total muscle mass. For fat measurements, only the thorax was used as this is the place where metabolic lipids used for flight are located (Marden 1989). Males’ thorax was placed in a desiccator, and following the protocol of Plaistow and Siva-Jothy (1996), fat was extracted via chloroform immersion. The thoracic weight was recorded (in grams) previous to and after the extraction. The difference between both measures was interpreted as total thoracic fat (fat load). Body size in those males that engaged in contests was measured as forewing length (in millimeters), from the base of insertion to the thorax to the wing tip. Body size from the experiment was measured as head (eyespan) width (in millimeters). For both size measurements, we used a digital calliper (precision  $\pm 0.01$  mm).

## Statistical analyses

### *Ages of contesting males, fat load, and muscular mass at different ages and status*

Ages of contesting males belonging to the first male set were compared using contingency tables. For the second set, we compared both fat load and muscle mass of the 3 age male categories using nonparametric tests for analyses of variance because data were not normally distributed (using Kolmogorov–Smirnov tests) and not suitable for transformation.

### *Contest outcome*

Data were normally distributed (using Kolmogorov–Smirnov tests); therefore, it was not necessary to perform data transformation prior to analyses. We used binary logistic regression to investigate whether 1) spot area, 2) spot red chroma, 3) spot brightness, 4) thoracic red chroma, 5) thoracic brightness, or 6) wing length best predicted contest winning or losing males (male status). We constructed a model in which male status was the dependent variable and spot area, spot red chroma, spot brightness, thoracic red chroma, and wing length were covariates. We used the logit link function to specify the binomial error structure. Furthermore, we tested all pairwise interaction terms between covariates, but none of these were statistically significant ( $P > 0.273$  in all cases); therefore, they were removed from the analysis.

Similarly, we used binary logistic regression to investigate whether fat load, muscle mass, or body size or combination thereof best predicted contest winning or losing males (male status). In this model, male status was the dependent variable and fat load, muscle mass, and wing length were used as covariates. We tested all pairwise interaction terms, but they were not statistically significant ( $P > 0.142$  in all cases); therefore, they were not further considered. The binomial error structure was specified using a logit link function.

### *Condition dependence experiment*

With the exception of fat load, all data were normally distributed (using Kolmogorov–Smirnov tests). Fat load was adjusted to a normal distribution using the formula  $\sqrt{x} + \sqrt{x+1}$  (Zar 1999). We investigated differences in 1) spot area, 2) spot red chroma, 3) spot brightness, 4) muscle mass, and 5) fat load between experimental and control males (treatment) using 5 general linear models. We only used these traits given that our contest results suggested that spot area—but not spot red chroma, spot brightness, muscle mass, or fat load—was an ornamental trait (see Results). In these models, we used either of the variables mentioned above as the dependent variable, treatment was used as a factor, and head width was used as a covariate. The interaction term treatment  $\times$  head width was tested in each model, but it was not significant ( $P > 0.147$  in all cases); therefore, we did not consider these terms in further analyses.

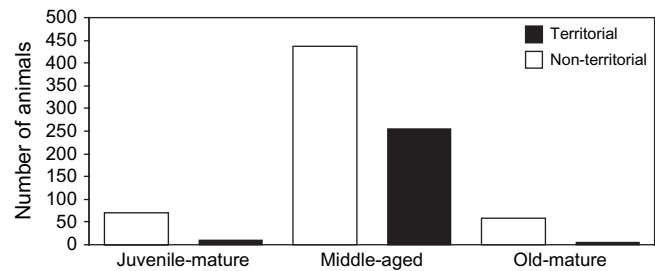
All statistical analyses were conducted using SPSS (version 15; Chicago, IL) setting an alpha value of 0.05.

## RESULTS

### **Ages of contesting males, fat load, and muscular mass at different ages and status**

Using the first set of males, we found that middle-aged males were most frequently engaged in contests compared with the other age categories ( $\chi^2 = 45.55$ ,  $P < 0.0001$ ; Figure 2).

For the second set, territorial males were only middle aged ( $N = 30$ ), whereas nonterritorial males were juvenile mature ( $N = 14$ ), middle aged ( $N = 30$ ), or old mature ( $N = 15$ ). These numbers indicate that territorial males were basically



**Figure 2**

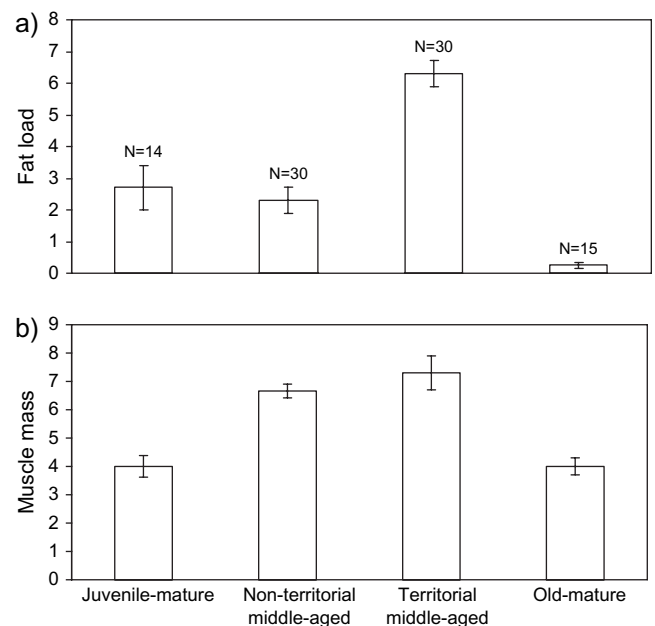
Frequency of males at different ages engaged in contests.

middle-aged animals. There were significant differences in fat reserves across males with middle-aged animals having the highest value (Kruskal–Wallis  $H = 46.15$ ,  $P < 0.0001$ ; Figure 3a). Similar results were observed for muscle mass (Kruskal–Wallis  $H = 33.56$ ,  $P < 0.001$ ; Figure 3b) in which middle-aged animals had the highest value.

### Contest outcome

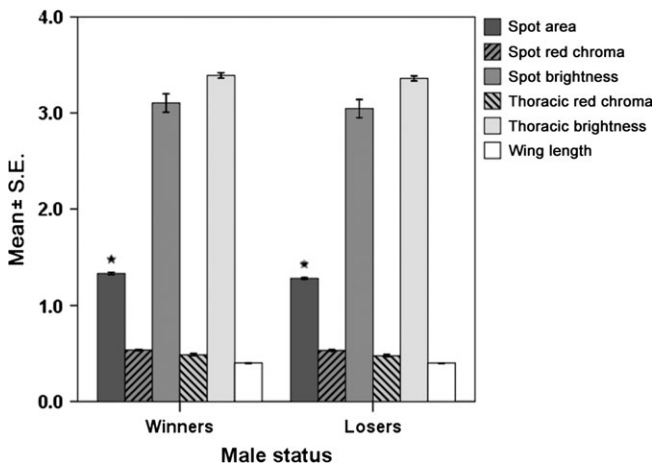
Males that won a contest were more likely to have a larger spot area than males that lost a contest ( $\chi^2_{1,18} = 4.909$ ,  $P = 0.027$ ; Figure 4). However, there were no differences in spot red chroma ( $\chi^2_{1,18} = 0.637$ ,  $P = 0.425$ ; Figure 4), spot brightness ( $\chi^2_{1,18} = 0.060$ ,  $P = 0.807$ ), thorax red chroma ( $\chi^2_{1,18} = 1.002$ ,  $P = 0.317$ ; Figure 4), thorax brightness ( $\chi^2_{1,18} = 0.835$ ,  $P = 0.361$ ; Figure 4), or wing length ( $\chi^2_{1,18} = 0.236$ ,  $P = 0.627$ ; Figure 4) between contest winning and losing males.

Winner males had a higher fat load than loser males ( $\chi^2_{1,22} = 4.828$ ,  $P = 0.028$ ; Figure 5); however, there were no differences in muscle mass ( $\chi^2_{1,22} = 2.765$ ,  $P = 0.096$ ; Figure 5) or wing length ( $\chi^2_{1,22} = 1.079$ ,  $P = 0.299$ ; Figure 5) between these males.



**Figure 3**

(a) Fat load (grams  $\times 10^{-3}$ ) and (b) muscle mass (grams  $\times 10^{-2}$ ) in relation to age and territorial status.



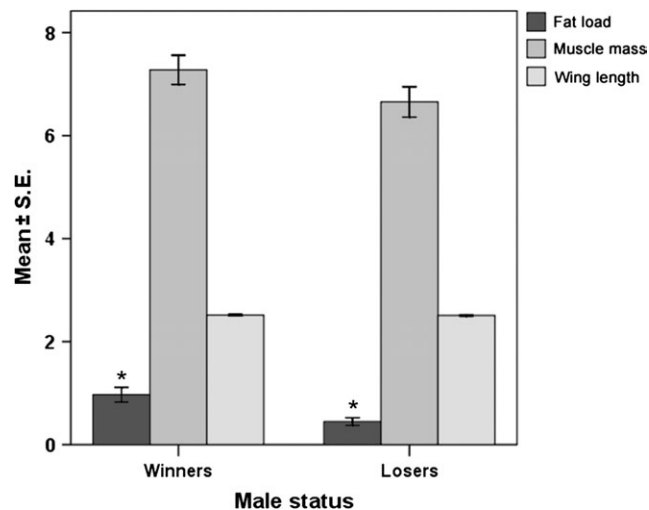
**Figure 4** Spot area, spot red chroma, spot brightness, thoracic red chroma, thoracic brightness, and wing length in contest winning and losing males. In order to facilitate visualization, all variables were  $\log_{10}$  transformed. Furthermore,  $\log_{10}$  spot red chroma and  $\log_{10}$  thoracic red chroma were given positive values. Note that the figure is consistent with our main results. Comparisons between contest winning and losing males were carried out using a binary logistic model. The only significant difference is denoted with asterisks.

**Condition dependence experiment**

Experimental males were more likely to develop smaller spot areas than control males ( $P = 0.001$ , Model 1, Table 1; Figure 6a). Experimental males had higher levels of spot brightness than control males ( $P = 0.050$ , Model 3, Table 1; Figure 6b). However, there were no differences in spot red chroma, muscle mass, or fat load among treatment males (see Table 1). Head width did not correlate with any dependent variable (see Table 1).

**DISCUSSION**

Fat reserves and muscle mass had the highest values in middle-aged males although this difference was more evident in terri-



**Figure 5** Fat load (grams  $\times 10^{-3}$ ), muscle mass (grams  $\times 10^{-2}$ ), and wing length (centimeters) in contest winner and loser adult males. Comparisons between contest winner and loser males were carried out using a binary logistic model. A statistically significant difference is denoted with asterisks.

torial males compared with nonterritorial males. Given that middle-aged males compete for territories most frequently and are actually the ones that defend a territory, the fat and muscle comparison at this age implies that these males are more physiologically prepared for territorial competition. This also indicates that there are restrictions that result in some males (i.e., the nonterritorial) not being able to reach high levels of these traits. Possibilities for these restrictions are as follows: 1) an inability to attain a large body size while foraging during the larval stage, which allows for the accumulation of more fat reserves during the teneral stage (Plaistow and Siva-Jothy 1999) and 2) difficulties during prey foraging in the teneral stage that affect fat production. Furthermore, we also found that both young and old males had very low fat and muscle levels. For young males, the explanation is that fat and muscle are still being constructed. For old males, these low values reflect a male that was never able to secure a territory because it did not achieve high fat reserves or an exterritorial male that is energetically exhausted. This latter suggestion would be in agreement with previous studies in this species, which indicate that, after losing a territory, males have extremely low fat values and that these values cannot be retrieved (Contreras-Garduño et al. 2006). This means that males cannot become territorial again, and so they are left aside in the territorial competition. Our results also indicated that only spot area correlated with winning a territorial contest. This result echoes previous results in this and other calopterygid species, in which males with larger pigmented wing areas defend territories and/or stay in these sites for longer (Grether 1996b, 1997; Hooper et al. 1999; Siva-Jothy 1999; Córdoba-Aguilar 2002; Contreras-Garduño et al. 2006; Serrano-Meneses et al. 2007). These males were thus better at defeating other males during territorial contests. These results, along with our age findings, suggest that only middle-aged males with larger spots are able to obtain a territory. Selection for energetic signaling would operate on these males only because young and old males rarely fight for territories. Despite having large spots, young males would not have acquired enough fat and muscle to fight, whereas older, large-spotted males would have already depleted their fat reserves. Given some recent results suggesting that American rubyspot males aggregate in a lek fashion (Raihani et al. forthcoming) and the presumable lack of precopulatory female choice (Grether 1996a), the signaling function would only be selected via male–male competition.

It is likely that males in better condition are able to produce larger pigmentation areas (Grether 1997). This better condition may be explained by the amount of dietary resources animals obtained prior to the development of pigmentation (for such evidence in another calopterygid, see Hooper et al. 1999), which includes not only the expression of pigmentation but also fat and muscle mass manufacture (Plaistow and Siva-Jothy 1999; Plaistow and Tsubaki 2000). This better condition explains why we found that winner males had more fat reserves than loser males, an account that had been somehow documented previously but only by comparing territorial and nonterritorial animals in this (Contreras-Garduño et al. 2006; Serrano-Meneses et al. 2007) and other calopterygid species (Marden and Waage 1990; Marden and Rollins 1994; Plaistow and Siva-Jothy 1996). However, we did not find support for the possibility that spot and thorax color properties had a role in signaling energetic reserves as documented by Fitzstephens and Getty (2000) for another calopterygid, *C. maculata*. Our present results are coherent with our previous findings in which we looked for differences in spot and thorax color properties with no differences in males according to status, age, and condition (as affected by diet and immune challenge) (Contreras-Garduño, Buzatto, et al. 2007). Although

Table 1

General linear models of spot area (Model 1), spot red chroma (Model 2), spot brightness (Model 3), muscle mass (Model 4), and fat load (Model 5) of experimental and control teneral males

Model	Dependent variable	Mean $\pm$ SD	Error df	Factor		Covariate	
				Group	P	Head width	P
Model 1	Spot area	14.58 $\pm$ 3.32	13	49.072	0.001	0.260	0.619
Model 2	Spot red chroma	0.36 $\pm$ 0.01	13	0.293	0.597	0.436	0.520
Model 3	Spot brightness	1897.85 $\pm$ 146.55	13	4.649	0.050	0.823	0.381
Model 4	Muscle mass	0.0015 $\pm$ 0.0008	11	1.373	0.266	0.014	0.908
Model 5	Fat load	1.0277 $\pm$ 0.0200	11	1.174	0.302	0.349	0.567

All nonsignificant interaction terms group  $\times$  head width were removed from the models (see Materials and Methods). df, degrees of freedom; SD, standard deviation.

these interspecific differences are discussed in detail by Contreras-Garduño, Buzatto, et al. (2007), we could only mention here that these may be due to pigment formation and function as both species differ in pigmentation color (*C. maculata*: green and blue; *H. americana*: red) and behavior (*C. maculata* males court females, whereas *H. americana* males

do not). In evolutionary terms and in short, it makes more sense that color pigmentation may be maintained via precopulatory female choice in *C. maculata*, which may not be the case for *H. americana* (Contreras-Garduño, Buzatto, et al. 2007).

We used the information that spot area may be an ornamental trait, unlike other spot color, fat load, and muscle mass characteristics, to compare how sensitive ornamental and nonornamental traits are to environmental stress. A review by Cotton et al. (2004b) determined that only a handful of studies fell into the category of properly designed protocols that have tested this idea. Although these studies are not contradictory to this idea, they are relatively few to claim a general pattern. Cotton et al. (2004b) suggested 3 methodological actions for doing this test: 1) the comparison of ornamental versus nonornamental traits (traits under weak or no sexual selection pressure), 2) control of body size as this trait is often correlated with ornamental traits and so these latter traits may communicate information other than body size, and 3) a varying degree of stressful regimes, as this may simulate a more realistic situation in nature.

In the present study, we were not able to demonstrate the last point but nevertheless our work still sheds light on this evolutionary issue. In particular, we only provided 2 stressful regimes, one with no monofilament and one with a single monofilament, because an increased challenge (e.g., 2 monofilaments at the same time) could kill the animal within a few hours (Contreras-Garduño J, unpublished data). As previously mentioned, it would have been desirable to include other nonornamental traits but many possible candidate traits are difficult to handle in teneral males. Nevertheless, our results are still compatible with what Cotton et al. (2004a) have found, in that ornamental traits are more sensitive to stress than nonornamental traits. According to our experimental setting, the immune challenge may have imposed a resource allocation conflict. Perhaps, the resources devoted to immune defense negatively affected spot area development. Siva-Jothy (2000) has argued that there is a link between gregarine immune defense and black pigmentation in the wing in *Calopteryx splendens xanthostoma* apparently because both functions rely on the same basis, melanin. It is unlikely that this is the case for the American rubyspot for several reasons: 1) instead of black, red pigmentation is present so that the basis for this color may be different and 2) gregarines are uncommon at least in the population we studied here (Córdoba-Aguilar et al. 2006). However, spot area correlates not only with the ability to cover with melanin a nylon monofilament but also with the phenoloxidase activity (an enzyme that plays a key role during immune defense; Söderhäll and Cerenius 1998; Sugumaran 2002) and hydrolytic enzyme (which participate in immune activation; e.g., Cheng 1992) activity and number (Contreras-Garduño

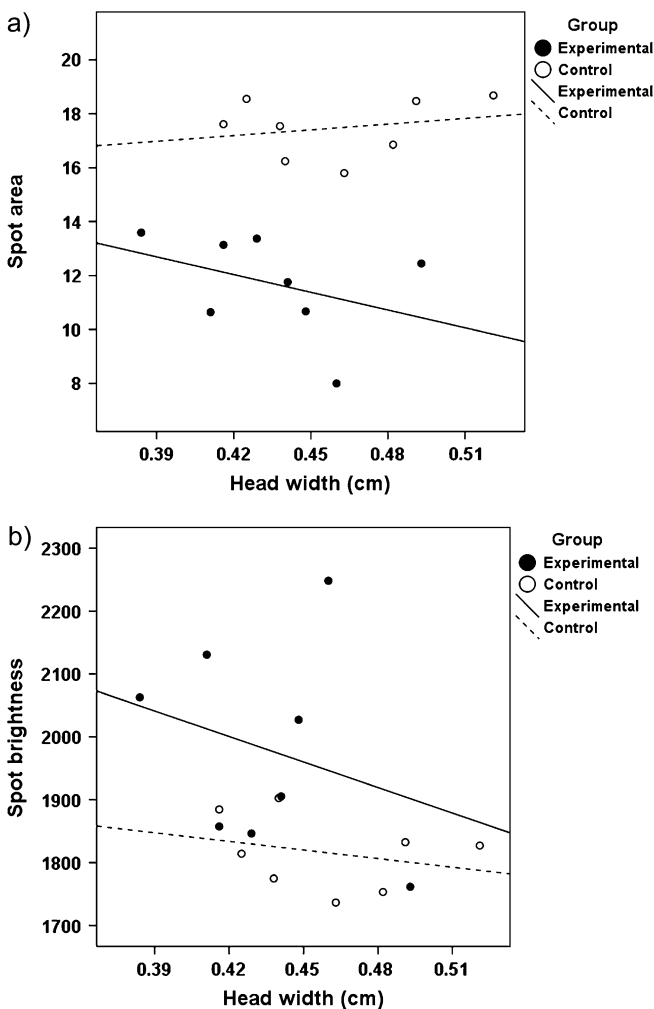


Figure 6 (a) Spot area (centimeters) and (b) spot brightness (nanometers) in relation to head width in experimental and control teneral males.

et al. 2006; Contreras-Garduño, Lanz-Mendoza, and Córdoba-Aguilar 2007), which further supports a link between red pigmentation and general immune ability. Unpublished results suggest that the juvenile hormone mediates resource allocation to immunity and several traits in this species (Contreras-Garduño J, Córdoba-Aguilar A, unpublished data).

One paradoxical result is that there was an increase (rather than a decrease) in spot brightness in experimental males compared with control animals. One possible explanation for this is that brightness has an adaptive role, which, according to our observations and measurements of male contests, does not seem to be the case. Furthermore, our previous results indicate that this trait is not different in territorial and nonterritorial males, does not change during different adult ages, and is not affected by diet (Contreras-Garduño, Buzatto, et al. 2007). This suggests that spot brightness may not communicate current male condition. There may be other instances in which this trait is selected but at least in terms of precopulatory male–male competition, this does not seem to be the case.

In summary, our results using the American rubyspot indicate that spot area is a good indicator of fighting ability. This trait may be evaluated during territorial contests in middle-aged males, an age at which males are more likely to compete because fat, energetic, and muscle mass levels are at their highest peak. Spot area, which contributes to precopulatory male–male competition (Grether 1996a), when compared with other traits apparently not shaped by sexual selection, showed heightened condition dependence. Cotton et al. (2004a, 2004c) proposed that heightened condition-dependent ornaments evolved mainly by female choice, which, interestingly, is not the case of our study species. Whether this heightened condition dependence can be applied to traits also driven mainly by male–male competition in other species needs to be verified.

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