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Resource Defense Polygyny by *Hetaerina rosea* Selys (Odonata: Calopterygidae): Influence of Age and Wing Pigmentation

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Abstract

Current evidence suggests that in *Hetaerina* damselflies males exhibit lek mating system. In this study, in order to answer if the same occurs in *Hetaerina rosea* Selys, we manipulated vegetation substrates used as territories and quantified the number of visiting females, males defending territories and fight intensity. We also examined whether body size and wing pigmentation are selectable traits in male-male competition, and if age affects male territorial behavior. Our results showed that males with larger pigmented areas won more contests, independently of body size. Old males changed from territoriality to sneaking strategy. Contrary to other *Hetaerina* species, males of *H. rosea* do not display lek behavior, but defend resources according to the resource defense polygyny strategy.

Introduction

Across many animal taxa, males use alternative mating tactics as a way to maximize fitness. Usually two tactics are described in which some males engage in quick and furtive copulations with females, whereas other males monopolize mating with a large number of females by aggressively defending a place where females gather (Thornhill & Alcock 1983). In the resource defense polygyny, males are territorial and guard resources needed by females (Emlen & Oring 1977). In many insects, this is a common reproductive tactic, and well known examples are the damselflies belonging to the Calopterygidae family (Thornhill & Alcock 1983). One example is the genus *Calopteryx*, whose males defend resources in riverine sites with submerged vegetation where females oviposit (Córdoba-Aguilar & Cordero-Rivera 2005).

With respect to other Calopterygidae, the genus *Hetaerina* has been used as a model for studies on male territoriality and mating behavior. In this genus, the sites defended by males do not need to have plant substrates for oviposition, since females rarely oviposit on the resource defended by males (Raihani *et al* 2008). Indeed, in *Hetaerina americana* (Raihani *et al* 2008), *H. macropus* (Eberhard 1986), *H. miniata* (Lefevre & Muehter 2004), *H. vulnerata* (Alcock 1982, 1987), *H. titia* and *H. cruentata* (Córdoba-Aguilar *et al* 2009) males do not defend a resource for females. Experiments with manipulation of vegetation substrates that females use to oviposit in *H. vulnerata, H. americana, H. cruentata* and *H. titia* (Alcock 1982, 1987, Córdoba-Aguilar *et al* 2009) showed that the presence or absence of this resource do not affect male territorial behavior, female visitation or mating success. Thus current evidence suggests that *Hetaerina* males exhibit lek mating system, where males defend territories that contain no resource, and females visit males solely to mate; territories basically consist of sites for male exhibition (Córdoba-Aguilar *et al* 2009).

Inside the territories, *Hetaerina* males fight against other conspecific males (Alcock 1982, 1987, Eberhard 1986, Grether 1996 a,b, Lefevre & Muehter 2004).

Territorial behavior in this group consists of aerial contests, in which two or more males engage in gyro-like flights in mutual pursuit (e.g. Johnson 1962, Waage 1973, Alcock 1982, Lefevre & Muehter 2004, Günther 2006). The outcomes of these contests are influenced mainly by thoracic fat reserves (Marden & Waage 1990, Plaistow & Siva-Jothy 1996, Serrano-Meneses *et al* 2007), which are correlated with wing pigmentation (Córdoba-Aguilar 2002, Contreras-Garduño *et al* 2006, Serrano-Meneses *et al* 2007). Recently, males with larger pigmented wing areas were shown to win most contests (Contreras-Graduño *et al* 2008). Age might also influence male behavior, as fat reserves have been shown to decay with aging (Plaistow & Siva-Jothy 1996). Hence, old males have low fat reserves and are no longer able to acquire or defend a territory and adopt a sneaking strategy (Forsyth & Montgomerie 1987). If so, we could expect old males to lose and engage in shorter contests.

In the present study, we examined the mating tactics in *Hetaerina rosea* Selys, testing the hypothesis that, as shown for other *Hetaerina*, they exhibit lek mating system. Considering that tropical systems are very different from subtropical ones, and that *H. rosea* is one of the most common damselfly of the Brazilian Neotropical savannas, this species may be the perfect model to test differences in relation to reproductive tactics between South and North American species as predicted by Córdoba-Aguilar *et al* (2009). We adapted Alcock (1982, 1987) and Córdoba-Aguilar *et al* (2009) approach to test if substrate removal or addition can influence male territoriality, aggressiveness and female visitation. We performed behavioral observations on male-male and male-female interactions to characterize reproductive and territorial behavior. Since old males may change their reproductive tactic with aging (e.g. *Calopteryx splendens* Harris, Plaistow & Siva-Jothy 1996; *Calopteryx maculata* Beauvois, Forsyth & Montgomerie 1987), we also checked whether the same trend occurs in *H. rosea* by recording the behavioral differences between old and young males during aerial contests. Additionally, we examined if traits of males such as body size and wing pigmentation could influence the outcomes of male contests.

Material and Methods

Study site and species

Fieldwork was conducted from May to October 2008 in the Laureano stream (Figs 1a and 1b) at Universidade de São Paulo, Ribeirão Preto County, southeastern Brazil (S 21°9'58'' W 47°51'51''). The study area is about 2m wide, 1m deep with slow water flow: the borders of the stream are covered by a riparian forest.

Male capturing, marking and age determination

All males were net-collected from 10:00h to 15:00h, a period of time where this species is sexually active (De-Marco Jr & Cardoso-Peixoto 2004), and numbered in the right forewing with a permanent marker with care to avoid marking the wing pigmented region (Córdoba-Aguilar 1994, Grether & Grey 1996). In the observation on the influence of male age on behavior, before marking we classified the age of males according to Córdoba-Aguilar (1994) and Plaistow & Siva-Jothy (1996), as follows: (1) teneral: individuals with highly transparent and flexible wings with developing pigmentation (2) young: wings not so transparent and flexible as the teneral ones, body bright red, and (3) old: pale body coloration and rigid, yellowish and sometimes damaged wings. All males were released in the same location they were captured, right after marking and classifying their age. As in other studies, our manipulation apparently did not affect their behavior or caused any damage (e.g. Córdoba-Aguilar 1994, Plaistow & Siva-Jothy 1996).

Oviposition substrate manipulation

To examine whether *H. rosea* exhibit a lek mating system or resource defense polygyny, we manipulated the vegetation substrates in 21 selected territories along the stream from September 15 to October 17, 2008. The manipulation was performed according to two treatments, both preceded by a control phase. In the removal treatment, in territories with a resident male (N = 10) were found on the control phase (day one) and we recorded: a) number of males present in each territory, to determine if males guard territories independently of the presence of vegetation substrates; b) number of effective male-male aggressive encounters, to evidence if males fight against each other even though there is no resource to guard; and c) number of females visiting each territory, to check if the presence of vegetation substrates affects mating opportunities. On the following day, we completely removed from the territory the substrates used by females for oviposition (floating roots and debris, Fig 1c) and recorded the same variables as we did on the previous day.

In the addition treatment, territories where no male was found (N = 11) were characterized as undefended sites on the control phase and in this case we added substrates used by females, consisting of root masses identified as oviposition sites (Fig 1d). These substrates were extracted from a *Ficus* tree, consisting of a 30cm long stem and a 10 x 10cm root mass that was placed on the bottom of the stream in a way that the stem was supported by stream banks and the roots floated on the water. We recorded the same variables as above on the day before and on the day of the manipulation, right after substrate addition. If *H. rosea* males exhibit a lek mating

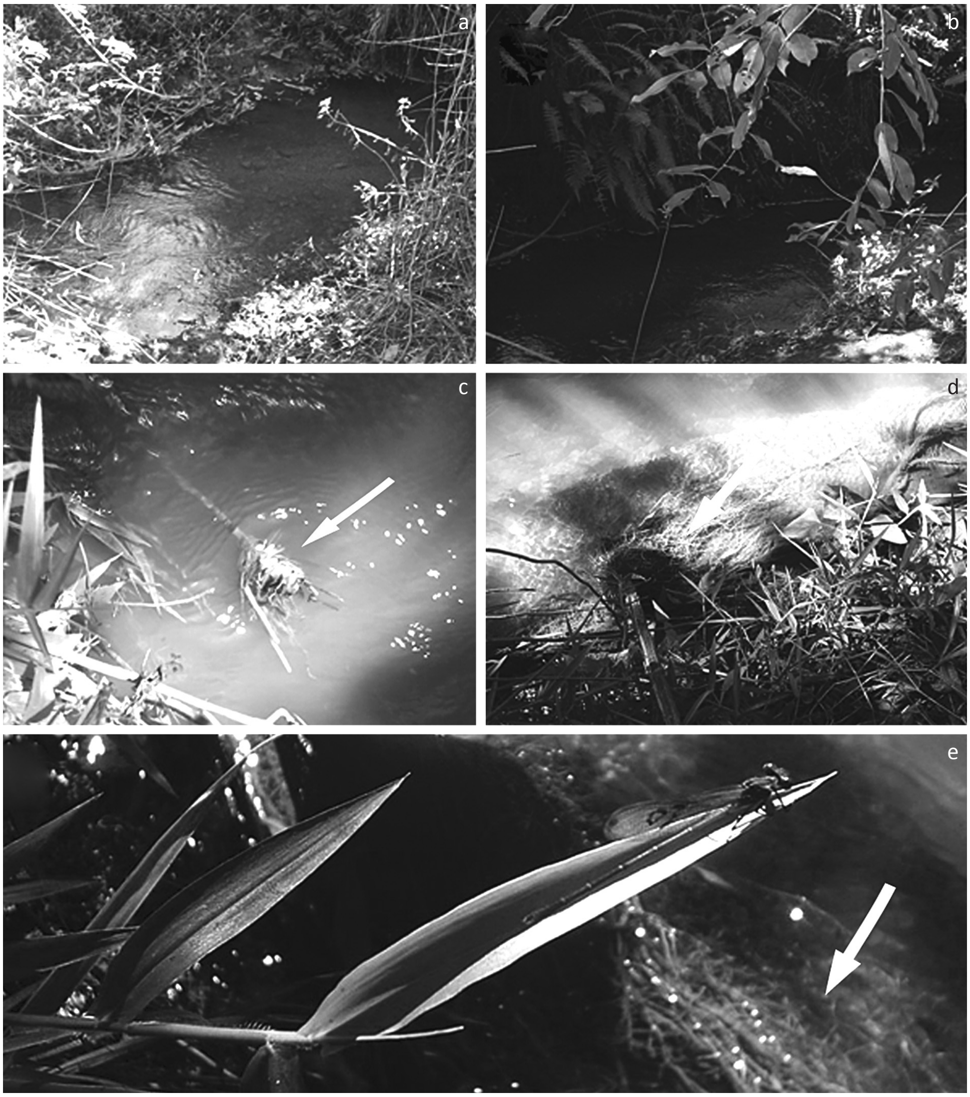

Fig 1 The stream at the study site (a and b) with floating roots and debris used as oviposition sites (c, white arrow); d) introduced oviposition site consisting of roots removed from a *Ficus* tree (white arrow); e) marked territorial male of *Hetaerina rosea* defending a mass of roots (white arrow) introduced at the study site.

system, substrate addition or removal should result in no significant differences between control and treatment phases, as well as significant differences would indicate that *H*. *rosea* males exhibit resource defense polygyny.

Contestants' characteristics

In another experiment, we collected winners and losers of ten individual contests. When a contest began, we noticed the outcome and collected the winner and the loser of the same contest. All wings were removed and photographed next to the body. Body size (measured from the head to the tip of abdomen, Córdoba-Aguilar *et al* 2009), total wing area and pigmented area of the four wings were measured using digital pictures (analyzed with Adobe Photoshop Extended CS3`s Measuring Tools®); each individual was photographed together with a ruler for scale patterning. Then, we calculated the proportion of the pigmented area in relation to the total wing area for all four wings, and the mean of each individual was used to compare wing pigmentation differences between winners and losers of each contest (Serrano-Meneses *et al* 2007).

Effect of age on male territorial behavior

To assess if *H*. *rosea* males were territorial, we made previous observations to address the question whether males fight against conspecifics and defend a particular site (Raihani *et al* 2008). To describe male territorial behavior and evaluate its relation to age, we first divided the study area in two 4m long transects across the stream, where we could observe two territories and the conflicting territorial males. To describe male-male interactions and to check if contest duration and outcomes could be influenced by the age of the participants, behavioral observations were made (*ad libitum* sensu Altmann 1974), alternately between the two transects. Observations lasted 30 min in each transect, focused on the contest duration, regarding the age of the participants. When a contest began we used a digital watch to record time duration of each contest. In addition we recorded the contest outcome as follows: the first male to leave the fight was considered the loser, and the winner the male that remained flying or perched on the site. We also recorded every male-female interaction to assess mating and female behavior inside territories.

Statistical analysis

Data were transformed using the formula √(x + 1) in order to meet normality and the values expressed in percentages were transformed using the formula arcsine √x. To compare the number of territorial males; male-male aggressive encounters and frequency of female visitation in territories before and after substrate manipulation, and to compare body size and wing pigmented area of winners and losers of contests, we used paired t-tests. To compare contest duration between young male fights and fights with old males participating, we used the t tests.

Results

Oviposition substrate manipulation

Substrate removal in natural territories that previously had owners significantly decreased the number of males at these territories and consequently the number of contests and females (Table 1). When substrates were introduced in areas that previously had no male defending it, the number of males, fights and visiting females at these territories significantly increased (Fig 1e, Table 1).

Contestants' characteristics

Larger males did not win more contests than the smaller ones (t = -1.3914; P > 0.05; N = 10). However, we found evidence that winners had a larger wing pigmented area compared to losers (t = 3.5057; P < 0.0066; N = 10; Fig 2a).

Effect of age on male territorial behavior

Young males usually defended and fought for territories, while old males sneaked through territories always avoiding contests with territorial males. Aerial contests were divided in long and short contests. Long contests occurred when two young males met each other; when an old male was one of the participants, the chase was significantly shorter (t test, t = 11.95; P < 0.0001, Fig 2b). Old males always lost contests and retreated to their last perch while the resident young males returned to patrol the site. No teneral male was seen engaged in a contest. The contests usually consisted of short chases (linear flights, lasting less than 5s) or aerial contests (circle-like flights varying in duration, Fig 3).

Behavioral observations

With a visiting female in a territory, two distinct behaviors were observed. The most common consisted of females flying short distances (approximately 50 cm) and going back to their perch (N = 84); males usually ignored their presence. If a male flew towards a female, she either chased (N = 5), rejected him by wing displaying (N = 8) or escaped from him when he attempted to grab her (N = 9). Mating was also observed (N = 7). In this case, the male rushed to the female and grabbed her almost immediately assuming the tandem position. After copulation, the couple flew to a substrate (floating root masses) where the female was released by the male that perched nearby. The females laid eggs on the defended root mass, sometimes submerging to do that (N = 2).

Discussion

Hetaerina rosea is a territorial damselfly, where young males with larger pigmented areas on wings win more contests for preferred oviposition sites. Unlike other *Hetaerina* species, *H. rosea* males defend vegetation substrates and take the females to oviposit on the defended resource, characterizing a resource defense polygyny strategy (Emlen & Oring 1977). In Calopterygidae, e.g. *Calopteryx*, territoriality based on resource defense polygyny commonly occurs (Córdoba-Aguilar & Cordero Rivera 2005). However, Raihani *et al* (2008) and Córdoba-Aguilar *et al* (2009) suggest that *Hetaerina* males show a lek mating system. In lekking odonates, after mating the couple need to search for an oviposition site and is commonly harassed by multiple males (Córdoba-Aguilar *et al* 2009). The intense harassment incurs costs, mainly

Table 1 Number of males, fights and female visitation (mean ± SE), observed before and after removal or addition of substrate in male territories. Paired t-test was used for all comparisons.

	Addition (N = 11 territories)		Removal (N = 10 territories)		P
	Before	After	Before	After	
Number of males	0	2.0 ± 0.30	3.1 ± 0.40	0.6 ± 0.22	< .0001
Contests	0	1.5 ± 0.41	3.5 ± 0.68	0	-
Female visitation	0	1.0 ± 0.38	4.6 ± 0.70	0.6 ± 0.33	< .0001

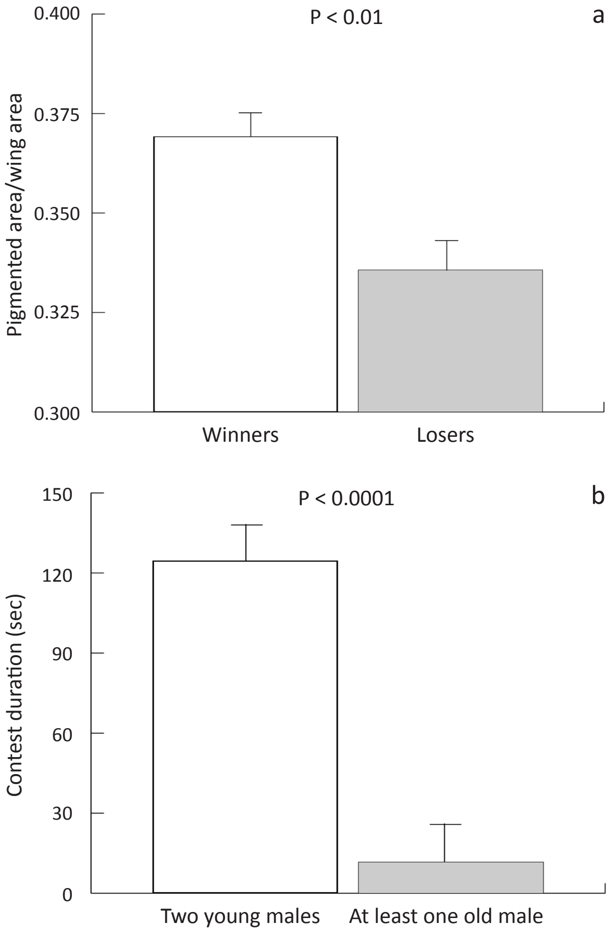

Fig 2 a) Territorial contests between males of *Hetaerina rosea* (Calopterygidae) as a function of wing pigmentation (paired t test), b) Contest duration as a function of male age (t test). Results are shown as mean ± SE.

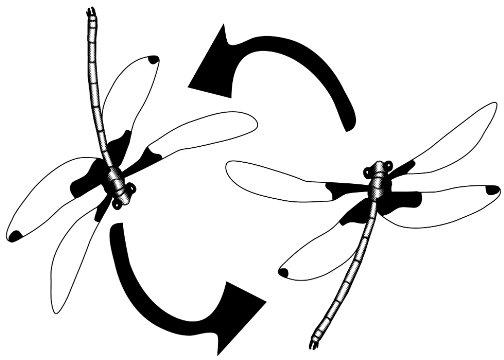

Fig 3 A typical aerial contest between two males of *Hetaerina rosea*.

to females (Córdoba-Aguilar 2009). Thus, it is expected that selection should act to decrease the costs, for example, favoring territorial males to hold a resource and attract females to oviposit on the defended resource, avoiding additional costs in searching for it. We suggest that in *H. rosea*, the evolution of male territoriality may thus reduce female harassment, an impacting additional cost common in lek strategists.

Differently from *Calopteryx*, there is no pre-copulatory courtship in *Hetaerina* (Córdoba-Aguilar 2009). In *H. rosea*, the females perch at the territories and keep hovering and returning to the same perch, probably inviting males to mate. Additionally, the fact that *H. rosea* females may reject males suggest that females may choose mates. This fact may support the hypothesis that female choice may influence male competition (Cox & LeBoeuf 1977). However, it has been suggested that female rejection may be a strategy of harassment avoidance instead of a mate choice strategy (Córdoba-Aguilar 2009).

In *Calopteryx*, males court females by hovering in front of them and female mating choices are influenced by male wing pigmentation (e.g. Siva-Jothy 1999). It has been demonstrated that in *Hetaerina*, the size of the pigmented area of the wings is under selection, mainly with respect to male-male agonistic displays (Grether 1996a, Serrano-Meneses *et al* 2007). The size of these pigmented areas are positively correlated with thoracic fat reserves (Contreras-Garduño *et al* 2006, Serrano-Meneses *et al* 2007, Contreras-Garduño *et al* 2008), which are used as fuel to flight (Marden & Waage 1990). Our results showed that males with a larger pigmented area won more contests, independently of body size (but see also Serrano-Meneses *et al* 2007). Serrano-Meneses *et al* (2007) suggest that, in the subtropical *H. americana*, large males can be more efficient in building up fat mass. However, in tropical areas the high density of preys may reduce the selective pressures on body size, as both large and small males have enough resources to build their fat body. So, the advantage of the larger male cannot be a rule for all species. In flies (Pie & Del-Claro 2002) and other odonates (Serrano-Meneses *et al* 2008) for example, smaller males can be more aggressive and agile than larger ones, winning more contests.

Forsyth & Montgomerie (1987) suggested that in *C. maculata,* sneaking is a "make the best of a bad situation" tactic adopted by older males when intense male-male competition forces them to abandon territoriality. Sneaking and stealing females from another males` territories would allow males to prolong their reproductive lifespan to a period when they will be no longer able to hold a territory. Plaistow & Siva-Jothy (1996) also observed a relationship between territoriality and age in *C. splendens xanthostoma* Charpentier. These authors reported that older males have lower fat reserves and, when forced to get out of a territory, they become post-territorial and are no longer able to acquire a new territory and so must mate with females ovipositing on already defended resources. Our results are similar and corroborate Forsyth & Montgomerie (1987) and Plaistow & Siva-Jothy (1996), since old males in *H. rosea* rarely engaged in aerial contests, avoiding fights and always losing contests to territorial males (e.g. Plaistow & Siva-Jothy 1996). Older males of *H. rosea* also sneaked through younger male territories, perching near the water surface and waiting for an opportunity to mate.

Resource defense by males depends on the spatial and temporal distribution of mates, competitors and resources (Emlen & Oring 1977). Córdoba-Aguilar *et al* (2009) suggest that territoriality would be uncommon in species of arid environments, where males have few opportunities to guard resources. But, in tropical environments, resources like perches or oviposition sites are abundant and could favor territory establishment (Córdoba-Aguilar *et al* 2009). Our results corroborate this hypothesis. In tropical regions, vegetation is commonly dense, thus we may expect the habitat of *H. rosea* to be more heterogeneous and thus offers more potential territories to males. Environmental characteristics may be an important component involved in the selective forces that directed *H. rosea* to a resource-defense polygyny strategy, contrary to related species that live in more depleted habitats.

Studies on the behavioral ecology of tropical damselflies, mainly in South America, need to be encouraged (e.g. Del-Claro & Torezan-Silingardi 2009). Similar to what we observed to *H. rosea*, other tropical species (Elpino-Campos *et al* 2001) can diverge from its temperate and subtropical related taxa, giving thus a better understanding of the evolutionary process molding the characteristics of each group and how they are affected by geographic, latitudinal and historic traits.

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