

**IMMUNE MELANIZATION ABILITY  
AND MALE TERRITORIAL STATUS  
IN *ERYTHEMIS VESICULOSA* (FABRICIUS)  
(ANISOPTERA: LIBELLULIDAE)**

A. CORDOBA-AGUILAR\* and V. MÉNDEZ

Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275,  
Circuito Exterior s/n, MX-04510 Ciudad Universitaria, México, D. F., Mexico

\* [acordoba@ecologia.unam.mx](mailto:acordoba@ecologia.unam.mx)

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Using a nylon filament implant inserted in the thorax, it was tested whether there were immune ability and size differences between territorial and nonterritorial ♂♂ that gather in lentic aquatic sites. It was found that territorial ♂♂ mounted a larger melanin-based immune response than nonterritorial ♂♂. This is coherent with current results in other odon. and is interpreted as territorial ♂♂ being in better condition than nonterritorial ♂♂. However, there was no size difference between the territorial and nonterritorial individuals. This suggests that size may be a poor predictor of immune ability.

**INTRODUCTION**

One of the most recent emerging fields in ecological theory is that of ecological immunity. The main aim of ecological immunity is to understand the underlying forces that give rise to and maintain variation in immunity as well as the role of immunity in determining individual's fitness (ROLFF & SIVA-JOTHY, 2003). A basic tenet in ecological immunity theory is that immune responses are costly to produce. One of the physiological costs is how the animal would divert resources to the immune function and other functions (ROLFF & SIVA-JOTHY, 2003). Given this potential conflict of resource allocation, only some individuals could afford diverting resources to immunity.

Due to their suitability for manipulation, one of the animal groups that have best served for ecological immunity ideas is that of insects. Insect immunity relies on innate responses that do not apparently recognise a particular invader but exerts a generalised response (BOMAN, 1998). With fairly large invading agents, a

series of haemolymph cells of the invertebrate immune system adhere to the intruder encapsulating it. The encapsulation process is effected by a series of enzyme reactions which ultimately induce the cells to become melanized. Due to the rigid properties of melanine and once encapsulated, the invader is prevented from further development and is eventually eliminated (LAVINE & STRAND, 2002).

Recent studies in insect behaviour have suggested a link between sexual activities and immune function. In damselflies, for example, females that have recently oviposited have less intense immune responses, in the form of melanine production, than non-ovipositing females (SIVA-JOTHY et al., 1998). Possibly there is a mechanistic link between the effort of laying eggs (females submerge into the water during this behaviour) and melanine production that compromises the animal to divert more energy to one function than to other in situations of environmental stress.

In this paper we provide evidence of differences in melanine production after a non-pathogenic challenge in territorial and nonterritorial males of the dragonfly *Erythemis vesiculosa*. Males of this species gather at aquatic, lentic body waters that females use for oviposition. Males defend these places against conspecific males by chasing them out of the territory (ACA & VM, pers. obs.). Similar to other odonates (for a review see CORBET, 1999), a number of males are not able to defend a territory and, instead, wander trying to locate a female and, consequently, end up with a lower mating success than territorial males. For the activation of the immune response, we used a nylon filament which is a standard technique in immune studies (e.g. WIESNER & GOTZ, 1993). We also investigated whether there was a difference in size in males using different territorial tactics and to see whether it was related to immune ability.

#### MATERIAL AND METHODS

A large population of *E. vesiculosa* was studied near the marshes at Palo Verde National Park, Guanacaste, Costa Rica on 4 and 5 June 2004, from 11–14 h, the time at which dragonfly activity was more pronounced. This species inhabits ponds of variable size covered with vegetation. Fourteen territorial and 14 nonterritorial males were collected. The criteria for identifying a territorial male was that the individual chased away conspecific males and remained in the same aquatic place for several minutes (ca 10 min). A nonterritorial individual was away from the aquatic sites and, therefore, did not defend a territory. Animals were taken to the laboratory in glassine envelopes and within a black box to reduce insects' activity. Seven males of each behavioural status were immune challenged using a 2 mm length nylon filament which was inserted in the central, lateral region of the second thoracic sternite. Previous to insertion, a small hole was gently made with a piece of forceps so that the filament insertion was easier to be done. The filament was directly inserted in the thoracic muscles and left there for 8 hours, a time that has been documented as the minimum for completing an immune response in odonates (ACA, pers. obs.). During this time and to reduce the animals' activity (so that immune activity does not become compromised with other potential energetic-exhausting activities), the animals were left in the glassine bags and within a dark container. They were then preserved in ethanol (70%) and then the filaments carefully removed using dissecting forceps. A picture of each filament was obtained using a dissecting microscope and a digital camera. These pictures were stored

in a computer and the images projected on the computer screen. A sheet of gridlines of 5×5 mm was then put on the screen. We counted the number of total squares that the filament and covering melanine filled in and the number of squares of the covering melanine (the criteria was that if more than half of each square was filled, then it counted as one square). The relative area covered by melanine was then obtained taking melanine and the filament as 100%.

The 7 males remaining for each behavioural status were measured (in cm) from the basis to the tip of the right, anterior wing. We compared both immune responses and size of both territorial and nonterritorial animals. Means  $\pm$  STD are provided unless stated otherwise.

## RESULTS

Immune response was higher for territorial males ( $0.88 \pm 0.29$ ,  $N = 7$ ) than nonterritorial males ( $0.47 \pm 0.23$ ;  $t$ -test =  $-4.04$ ,  $P = 0.001$ ,  $N = 7$ ; Fig. 1). In contrast, however, there was no difference in size according to male behavioural status (territorial,  $3.97 \pm 0.21$ ; nonterritorial,  $3.97 \pm 0.19$ ;  $N = 7$  each;  $t$  test =  $0.072$ ,  $P > 0.05$ ).

## DISCUSSION

Previous studies in Odonata have already detected differences in immune responses depending on the behavioural status and reproductive activities (e.g. SIVA-JOTHY et al., 1998; SIVA-JOTHY, 2000; RANTALA et al., 2000; KOSKIMÄKI et al., 2004). These studies have used a rationale of a cost being paid by the different tactics using an energetic trade off scenario. For example, studies of aggression in damselflies have suggested that melanin production gets reduced after long lasting, male-male encounters and males using a territorial tactic usually have also better immune responses (KOSKIMÄKI et al., 2004). Since one of the costs of fighting is a reduction in fat stored in the thorax (where the wing muscles are inserted), somehow the reduction of fat also affects immune ability. Our results are in agreement with these other studies: males that are occupying the territories are better immuno-competent than nonterritorial males. Possibly, only males in good condition are able both to defend a space and deal with pathogens, an ability that nonterritorial males do not have. The physiological basis of this relationship may lie on the reserves that males have. It is likely that nonterritorial males are fat exhausted (e.g. old males) or

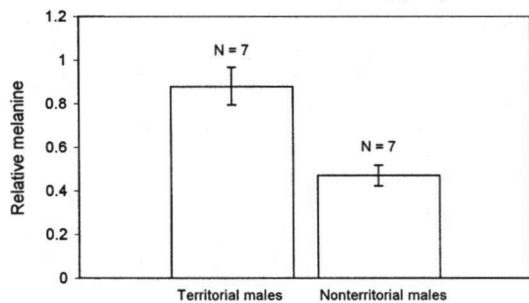


Fig. 1. Melanine quantification (mean  $\pm$  SE) of an immune filament challenge in relation to territorial status in *Erythemis vesiculosa*.

in the process of manufacturing this (e.g. young males) which means that have not accrued enough resources which may also be used for immune responses. Possibly, fat directly affects melanine production so that animals with less fat are unable to produce a good immune response. The relationship between fat and immunity, however, may not be direct and both may be a consequence of a reduced income of food that the animal has obtained. A support to this is that in beetles, recently-fed animals increased their immune responses significantly (SIVA-JOTHY & THOMPSON, 2002).

The absence of size effects in our comparison suggests that this trait is not an indicator of immune ability and male territorial status. In territorial odonates, recently emerged males are fat depleted so that they start building up resources for reproductive activities (e.g. PLAISTOW & SIVA-JOTHY, 1999). In males, fat is used to obtain and defend a territory. Once the male has obtained enough fat, possibly, there is a positive relationship between size and fat reserves (larger size, more fat reserves). The fact, however, that fat gets reduced with time and decreases substantially when the animal is defeated during territorial contest (e.g. PLAISTOW & SIVA-JOTHY, 1996), makes that this positive relationship disappears. Since some large but old males that were once territorial will end up with less fat, means that size becomes a poor predictor of territorial status. Given this and the relation of food consumption and immunity in insects, suggests that size may not be a good predictor of immune response either.

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