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Is there a trade-off between the melanin allocated to the immune system and to camouflage on larvae of the dragonfly *Micrathyria catenata* Calvert,1909 (Odonata: Libellulidae)?

Existe uma demanda conflitante entre a melanina alocada para o sistema imune e para a camuflagem em larvas da libélula *Micrathyria catenata* Calvert,1909 (Odonata: Libellulidae)?

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Abstract

In insects, the immune system responds to the presence of antigens involving them in melanin. However, the melanin is also allocated into the exoskeleton's pigmentation, used to camouflage. We aimed to test the existence of a trade-off between the allocation of melanin to the immune system and to camouflage on the larvae of *Micrathyria catenata*. We conducted the study in the "Reserva do km 41" (41 km' Reserve), 80 km distant from Manaus, Amazonas, Brazil. We implanted a nylon line into the abdomen of 30 larvae and observed if had or not deposition of melanin in the line. We counted the number of individuals who responded to implant depositing melanin and, later, we took photos of the larvae's heads and calculate gray intensity. We used a t-test for independent samples. 76% of larvae responded to treatment depositing melanin on the implants. There were no significant differences in the intensity of gray between the larvae that responded to the implants and those who did not responded. There is no trade-off to allocation of melanin for camouflage and for the immune system. This should happen because the immune system is not limited by the acquisition of resources or the camouflage's demand for melanin is not enough to influence the immune system.

Key words: dragonfly, camouflage, immune system, *Micrathyria catenata*, trade-off.

Resumo

Em insetos, o sistema imunológico responde à presença de antígenos encapsulando-os com melanina. Porém a melanina também é alocada no exoesqueleto para pigmentação, promovendo a camuflagem. Objetivamos testar se existe uma demanda conflitante entre alocar melanina para o sistema imunológico e para a camuflagem nas larvas de *Micrathyria catenata*. Realizamos o estudo na Reserva do km 41, a 80 km de Manaus, Amazonas. Inserimos um implante de nylon no abdome de 30 larvas e observamos se houve ou não deposição de melanina. Contamos os indivíduos que responderam ao implante depositando melanina. Posteriormente, fotografamos as cabeças das larvas e calculamos a intensidade de cinza. Usamos um teste t para amostras independentes. 76% das larvas responderam ao tratamento depositando melanina nos implantes. Não houve diferença significativa na intensidade de cinza entre as larvas que responderam aos implantes e as que não responderam. Não encontramos uma demanda conflitante na alocação de melanina para a camuflagem

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e para o sistema imunológico. Isso pode ser devido ao fato de o sistema imunológico não ser limitado pela aquisição de recursos ou de a camuflagem não demandar melanina suficiente para influenciar o sistema imune.

Palavras-chave: libélula, camuflagem, sistema imunológico, *Micrathyria catenata*, demanda conflitante.

Introduction

Infections in insects stimulate innumerous immunological responses that involve pathogens' recognition by receptors and reactions that produce chemicals signals responsible for changes in genes' defensive expression (Schmid-Hempel, 2005). These signals promote the activity of the hemocytes, which are specialized cells that promote phagocytosis or encapsulation of foreign bodies through the deposition of melanin (Gillespie *et al*., 1997). Although immunocompetence offers an obvious benefit, reducing infestation by parasites, there is often a trade-off with other fitness' components (Schwartz and Koella, 2004). When the defense is more expensive than its benefits (e.g. protection in the absence of parasites) it should happen a re-allocation of resources from immune system's maintenance to others functions of the body (e.g. reproduction) (Rigby *et al.*, 2002). Freitak *et al*. (2003), studying the butterfly *Pieria brassicae* Linnaeus, 1758, and Schwartz and Koella (2004), studying the mosquito *Aedes aegypti* Linnaeus, 1762, showed that there is not a general cost of immunity, because this cost varies depending on the type of antigen. Those antigen recognized by the immune system as potentially harmful encourage a faster and effective response, that is also more expensive to the organism (Schwartz and Koella, 2004).

In insects, there may be a trade-off between the melanin allocated to the immune system and those used for camouflage. Vasconcellos-Neto and Gonzaga (2000) cited several examples of butterflies, spiders and booklices that use melanization of the body as a form of camouflage. A cryptic pattern can provide adaptive advantages for both prey, to escape from predation, and cryptic predators, to get closer to their prey to capture them (Krebs and Davies, 1996). In aquatic systems, for example, dragonfly's larvae are predators and the cryptic coloration, present by the formation of patches of melanin in the integument of its bodies, is extremely important to let these organism come close to their prey to capture them (Corbet, 1999).

Environments such a puddle may have a high abundance of larvae of many species of dragonflies. In Central Amazon, the *Micrathyria catenata* (Libellulidae) dragonfly's larvae are fairly common in standing puddles, frequently found foraging on submerged leaves near the margins. This species is a good object of study in immunological ecology due to its fast immunological responses by the encapsulation of antigens, such as experimental implants, with melanin.

We aimed in this study to test if there is a trade-off between the allocation of melanin to immune system and to camouflage in the larvae of *M. catenata*. Our hypothesis is that larvae that respond to antigens depositing melanin are less pigmented than larvae that not respond. We expect that larvae that deposit melanin on implants show a lower intensity of gray color in their integument.

Material and methods

Study area

We conducted this study in the "Reserva do km 41" (41 km' Reserve) of Biological Dynamics of Forest Fragments Project (2º26'25"S, 59°45'43"W), located about 80 km north from Manaus. The weather is warmth and humid, with two marked seasons, mean annual temperature is 26 ºC and annual rainfall varies between 1,900-2,300 mm (RADAMBRASIL, 1978). We collect the larvae from a permanent puddle located beside the road that crosses the forest. The puddle had an ellipsoid shape with approximately 15 m length and 6 m wide. The water column's maximum depth was 1 m, so that some dry branches that fall from beside the forest were partially exposed. Because its western margin was close to the forest, the puddle received direct sunlight only in the period between 8:00 and 14:00 h.

Data collection and analysis

We collected the larvae of *M. catenata* in the period of 9:00 to 11:30 h using a sieve. We put the individuals collected in a plastic jar with water also collected in the puddle. In each larva of *M. catenata* we inserted a nylon implant of 0.5 cm length on its right anterolateral portion of the abdomen. After the insertion of implants, the larvae were isolated during 24 h in Petri dishes containing water. After 24 h, we removed the implants and observed in stereoscopic microscopes whether it presented or not the deposition of melanin. Of forty collected larvae, only sixteen survived. Then, we collected twenty others larvae and repeated the experiment, but keeping them isolated for 12 h. Of these larvae, six died and we analyzed thirty larvae in total, sixteen under a 24 h regimen of implant and fourteen under a 12 h regimen of implant. We counted the number of individuals who responded to the implant depositing melanin on it.

To test the existence of a trade-off in the allocation of melanin between the immune system and to use in camouflage, we photographed the head of the larvae in dorsal view using a camera Canon Power Shot A570IS (4x optical zoom and 7.1 megapixels of resolution) coupled to the stereomicroscope. We used Adobe Photoshop 7.0.1 (Adobe Systems Inc.) to edit the photos as follows: we select the area of the head of the larvae, excluding the eyes, transformed the images into shades of gray and calculated the intensity of gray. The values supplied by the program ranges from 0 (completely black) to 255 (completely white). We subtracted 255 from the original's variable value to make it directly interpretable as shade of gray, indicative of melanin's quantity. To test if the implants with deposition of melanin showed differences in the intensity of gray, as evidence of investment in camouflage, we used the t-test for independent samples. To assess possible methodological problems related to the differences in the time of experiment in the test above, we evaluated the robustness of the results separately to the individuals with 12 h and 24 h of exposure in the same way as above, despite the smaller number of samples in the experiment at 12 h.

Results

Twenty-three (76%) larvae of *M. catenata*, of the thirty sampled, responded to treatment depositing melanin in the line implanted. Individuals who did not respond to the implant showed the intensity of gray values ranging between 181.7 and 212.2 (mean ± SD = 195.64 ± 12.53), while those who responded to the implants varied between 172.96 and 211.27 (mean ± SD = 192.28 ± 10.67). Considering all individuals, there were no significant difference in the intensity of gray between the larvae that responded to the implants and those who did not respond ($t = 0.701$, d.f. = 28, $p = 0.489$). The results were also not significant considering the 12 h ($t = 0.472$, d.f. = 12, $p = 0.645$) and the 24 h experiment ($t = 1.410$, d.f. = 14, $p = 0.180$).

Discussion

Siva-Jothy (2000) studied the damselfly *Calopteryx splendens xanthostoma* (Charpentier, 1825) and noted that there is a trade-off on the use of melanin pigmentation in the wings and to the immune system. Moreover, Schwartz and Koella (2004) found that the melanization of neutral implants had no effect on the fecundity of the mosquito *Aedes aegypti*. In this study, we did not find a trade-off in the allocation of melanin to the camouflage and to the usage into the immune system. We propose two hypotheses to explain this result: (i) the camouflage does not demand enough melanin to affect its use by the immune system; and (ii) well-fed larvae have enough power to supply both the demand of melanin for camouflage and for the immune system. According to Norris and Evans (2000), there is a trade-off between the immune system and components of life history only if resources limit the immune system. Rigby *et al.* (2002) affirms that when a food resource acquisition is low, the cost of resistance to parasites is due to the physiologic difficulty to re-allocate, to the immune system, this acquires resources, which would be used in other ways (e.g. eggs production and growth). However, a standard of camouflage that does not need much melanin may be enough to confer an adequate predation capacity to those larvae and also allowing the allocation of resources to immune system.

Schmid-Hempel (2005) states that the availability of resources, as well as the interference by parasites, affect the immune response of an organism. There is evidence that protein-rich food resources may limit the immunocompetence (Norris and Evans, 2000). If the preys of *M. catenata*'s larvae are abundant and nutritious, the larvae may have sufficient food resources to metabolize melanin to maintain both requirements for immune system and camouflage.

In the present study we did not quantify the immune response of larvae, we only classified as present or absent, as measured by the presence or absence of melanin deposited on the implants. This methodological limitation does not invalidate our results; however, it would be possible to have an accurate result if the melanin deposited on the implants was measured quantitatively. We suggest this approach in future studies that aim to examine the existence of a trade-off in the use of melanin by the immune system and camouflage in larvae of *M. catenata*.

The existence of a trade-off between immune system and components of the life history of animals is not a general pattern. In this study we demonstrate that there is not a trade-off in the allocation of melanin to the immune system and to camouflage dragonfly's larvae of *M. catenata*. This may be due to the immune system is not limited by the acquisition of resources or the camouflage does not demand melanin enough to influence the immune system. Future works should assess quantitatively the efficiency of the melanin uses into the immune system and other aspects of the life history of animals.

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