Original Article

MITOTIC ACTIVITY OF THE BRAIN GANGLIA IN DIFFERENT SUBPHASES OF THE LAST LARVAL INSTAR OF *MELIPONA QUADRIFASCIATA* LEPELETIER (HYMENOPTERA, APIDAE, MELIPONINI)

ATIVIDADE MITÓTICA DO GÂNGLIO CEREBRAL EM DIFERENTES FASES DO ÚLTIMO INSTAR LARVAL DE MELIPONA QUADRIFASCIATA (HYMENOPTERA, APIDAE, MELIPONINI)

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ABSTRACT: In recent years, the number of cytogenetic studies on *Melipona* species has increased considerably. However, most cytogenetic techniques used for these studies require preparations with a great number of metaphase cells for reliable analysis of the karyotypes. The present study seeks to evaluate which subphase of the last larval instar of *Melipona quadrifasciata* Lepeletier provides the greatest number of metaphases, which is here considered a direct measure of mitotic activity. A total of 25 defecating larvae were selected based on the quantity of feces in their intestines, so as to maintain five larvae in each of the five different developmental subphases. The brain ganglia of each larva were extracted and used for cytogenetic preparation. The number of metaphase mitotic cells per preparation was counted. An analysis of variance (ANOVA) model, with Tukey's post hoc tests, was conducted. It was observed that larvae in the second subphase, defined here as the subphase in which feces were visible below the segment VII, provided the greatest number of metaphases. Therefore, it is the most appropriate developmental subphase for cytogenetic preparations of brain glanglia in *M. quadrifasciata* and possibly in other *Melipona* species.

KEYWORDS: Cytogenetics. Karyotype. Metaphase. Stingless bees

INTRODUCTION

The first cytogenetic study on *Melipona* Illiger was performed by Kerr (1948), who found the chromosome number of 2n=18 for female *M. rufiventris* Lepeletier and *M. marginata* Lepeletier. Since then, several authors have worked with this genus. Currently, data is available in literature on the chromosome number, heterochromatin distribution and composition, and the localization of the nucleolus organizing regions for most of the 22 cytogenetically studied species of *Melipona* (ROCHA et al. 2007; LOPES et al. 2011).

However, knowledge on the cytogenetics of *Melipona* is far from being complete, because there is no data available for the other 23 described species. This is mostly due to methodological limitations in cytogenetic studies of the genus. Slide preparations with a large number of metaphase cells are needed for reliable karyotype analyses. Moreover, advanced cytogenetic techniques, such as FISH or those involving chromosome manipulation, are usually expensive (FERNANDES et al. 2011). Therefore, it is important to determine which tissue or organ has the highest mitotic activity, increasing the probability of obtaining a sufficient number of metaphases.

The brain ganglia is usually used for performing cytogenetic preparations of Hymenoptera, and it is important to know the time or time period which provides the greatest number of metaphase cells (IMAI, 1966; MENEZES, 1997, SOARES et al. 2004). Such information is currently not available for any *Melipona* species. In order to fill this lack of data, we sought to evaluate which developmental subphases of the last larval instar of *M. quadrifasciata* Lepeletier provided the greatest number of metaphases in cytogenetic preparations.

MATERIAL AND METHODS

We obtained larvae of *M. quadrifasciata* Lepeletier (Hymenoptera, Apidae, Meliponini) from a colony originally collected in a forest remnant in Viçosa (state of Minas Gerais, Brazil) and maintained at the apiary of the Federal University of Viçosa, at the same locality. Adult voucher specimens were deposited at the scientific collection of the apiary. Photographs of larvae (Figure 1A-E) were taken with a Nikon DS-Fi1 digital camera attached to a Nikon SMZ-745T stereomicroscope.

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We selected twenty five last instar larvae of *M. quadrifasciata* in order to have five larvae in each of five developmental subphases. Each subphase was defined by the quantity of feces observed in the digestive tract of the larvae (Figure 1A-E), as follows: A) feces visible above the VII segment; B) feces below the VII segment; C) feces below the VIII segment; D) feces below the IX segment; E) no visible feces. Cytogenetic preparations were made with the brain ganglia of larvae and mitotic chromosomes were obtained according to the methods proposed by Imai et al. (1988). After 24 hours we stained the slides with a Giemsa solution in Soerensen buffer (1:30; 0.06M; pH 6.8) for 20 minutes. The number of metaphases were counted in each slide preparation under an Olympus BX60 microscope, and this number was considered a direct measure of mitotic activity.

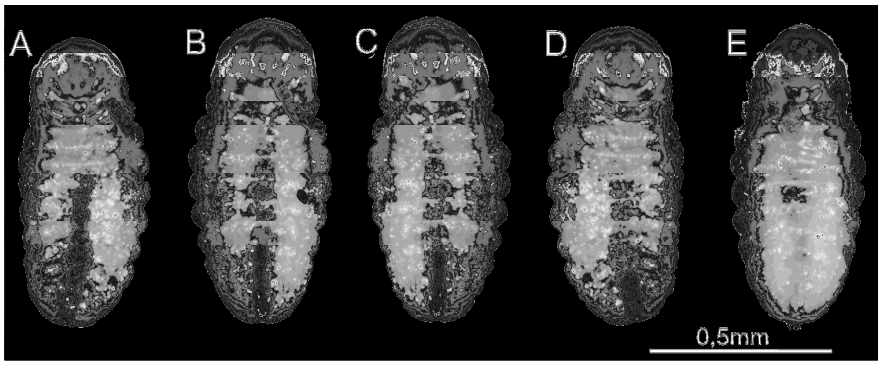

Figure 1. Classification of the last larval instar of *Melipona quadrifasciata* Lepeletier in five groups accordingly to the quantity of feces in their digestive tracts: A) feces visible above the VII segment; B) feces below the VII segment; C) feces below the VIII segment; D) feces below the IX segment; E) no visible feces in the digestive tract. Scale bar = 0.5 mm.

In order to evaluate which subphase provided the greatest number of metaphases, we conducted an analysis of variance (ANOVA), with Tukey's post hoc tests at 1% probability, in the software GENES (CRUZ, 2006).

RESULTS

The average number of metaphases per slide in the five subphases of the last larval instar is shown in Figure 2. The second subphase (Figure 1B), in which feces are visible below segment VII, showed the greatest average number of metaphases per slide (mean 104.8 ± 10.7), which was significantly different from the rest of the averages according to Tukey's test at 1% probability. The subphase in which the feces were found above the VII segment presented the lowest mean (31.4 ± 12.2), the following subphases did not present significant differences among themselves.

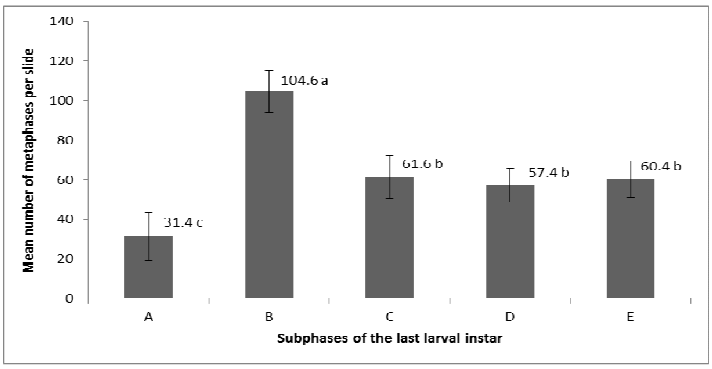

Figure 2. Mean number of metaphases per slide (y) in different subphases (x) for the last larval instar of *Melipona quadrifasciata* Lepeletier. A) feces visible above the VII segment; B) feces below the VII segment; C) feces below the VIII segment; D) feces below the IX segment; E) no visible feces in the digestive tract. Mean values followed by the same letters did not differ among themselves according to the Tukey test with 1% of probability; vertical whiskers represent the standard deviation.

DISCUSSION

Based on the results, we propose that the second subphase (Figure 1B) of the defecating larvae of *M. quadrifasciata* provides the greatest number of metaphases in preparations using brain glanglia, making it the most appropriate subphase for cytogenetic studies of the species. This is a very important result since it allows for avoiding unnecessary preparations, concentrating efforts on preparations using cells of the larval subphase which provide the greatest number of metaphases. It will certainly improve and facilitate subsequent cytogenetic studies of Meliponini and other Hymenoptera, which are increasing considerably in number and demand high quality which large amounts of chromossomic preparations (BARROS et al. 2009, GOKHMAN, 2009, MENEZES et al. 2011, GOKHMAN et al. 2011, CARDOSO et al. 2012).

Menezes (1997), in a study of the stingless bee *Tetragonisca angustula* Latreille (Meliponini), found the highest mitotic activity in larvae devoid of feces in the digestive tract, which correspond to the fifth subphase of the last larval instar in the present work. Moreover, there is no other available data on mitotic activity of Meliponini species. It is plausible to propose that close related species of Meliponini, mainly those grouped at a single genus, may have the highest mitotic activity in the same subphase. We recommend that future cytogenetic studies on other *Melipona* species shall focus on making preparations with the second subphase of the last larval instar, if a prior comparison among different subphases is not possible. In the case that a comparison is possible, it would be better to initially determine the subphase with the highest mitotic activity in order to obtain the greatest number of suitable metaphase cells. Imai (1966), working with species of Myrmiciane and Formicinae ants, showed that the prepupa was the subphase with the highest mitotic activity. The prepupa is the subphase just after the fifth subphase as defined here, and precedes pupation. Soares et al. (2004), working with the ant *Acromyrmex subterraneus subterraneus* Forel, showed that pupa had the greatest number of metaphases. The latter author also suggested that tissues with the highest mitotic activity contain large cells with large nuclei, where the neuroblasts are located in the corpora pedunculata of the protocerebrum.

Some parts of the body of the insects, such as the epidermis, show an increase in mitotic activity during ecdysis (CHAPMAN 1998). Ecdysis is controlled by the concentration balance of two hormones: the juvenile hormone produced by cells located in the corpora allata of the brain, and the ecdysone produced by prothoracic glands (CHAPMAN 1998). It appears that the relationship between these two hormones is responsible for controlling mitotic activity in several insect organs, including the brain. Future studies should evaluate if there is such a relationship and how it regulates mitotic activity in Meliponini.

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RESUMO: Estudos citogenéticos envolvendo o gênero *Melipona* vêm aumentando nos últimos anos. Entretanto, a utilização de várias técnicas para o estudo do cariótipo exigem preparações com um grande número de células em metáfase para uma análise confiável das características citogenéticas das espécies. O presente estudo teve como principal objetivo avaliar, para *Melipona quadrifasciata*, o instar do desenvolvimento larval mais adequado para estudos citogenéticos, no que se refere à atividade mitótica. Foram selecionadas 25 larvas defecantes divididas em cinco subfases de acordo com a quantidade restante de fezes no intestino. Os gânglios cerebrais das larvas foram extraídos e utilizados para a obtenção dos cromossomos mitóticos metafásicos. O número de metáfases por lâmina foi contabilizado para cada indivíduo e os dados submetidos à análise de variância (ANOVA) e ao teste de TUKEY. Foi observado que larvas da segunda subfase, definidas aqui como a subfase na qual as fezes se encontram na altura do VII segmento apresentaram o maior número de metáfases. Logo, esta é a subfase mais indicada para obtenção de grande número de metáfases em células do gânglio cerebral de *Melipona quadrifasciata* e, possivelmente, para outras espécies do gênero *Melipona.*

PALAVRAS-CHAVE: Citogenética. Cariótipo. Metáfase. Abelhas-sem-ferrão

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