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Oogenesis pattern and type of ovariole of the parasitoid *Palmistichus elaeisis* (Hymenoptera: Eulophidae)

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ABSTRACT

The knowledge on ovigeny in parasitoids is important for basic studies on physiology and applied biological control. The ovigeny pattern and type of ovariole of the parasitoid *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera: Eulophidae) were studied in newly-emerged females at seven, 14, 24 and 48 h intervals after their emergence from *Tenebrio molitor* L. pupae (Coleoptera: Tenebrionidae). Females of *P. elaeisis* presented ovaries composed by four ovarioles of the meroistic polytrophic type. The yolk accumulation and chorionogenesis in *P. elaeisis* were concluded 24 h after the female emergence. The 48 h-old females show a high quantity of egg ready for oviposition. These findings can help to improve the mass production of *P. elaeisis* and the augmentative biological control by using this natural enemy.

Key words: Biological control, morphology, natural enemies, ovigeny.

INTRODUCTION

Parasitoids are studied because of their importance in the biological control of pests and are used in the development of population models and interactions with their hosts in the field (Hassel 2000, Andrade et al. 2009, 2010a). Internal and external factors can influence the reproductive success and the strategy used by parasitoids, which are the basic components that should be considered for using these biological control agents (Ozkan 2007, Spataro and Bernstein 2007, Santolamazza-Carbone et al. 2007). The parasitism behavior depends on field conditions, host availability and the numbers of mature eggs during the cycle of each species (Jervis et al. 2001). For this reason, reproductive strategies results in variations on the dynamics of egg production by parasitoids (Ellers et al. 2000, Giron et al. 2004).

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The family Eulophidae has natural enemies and is used in many cultures (Santos Junior et al. 2006, Mendel et al. 2007) to control pest populations (Leite et al. 2006, Doganlar and Mendel 2007). *Palmistichus elaesisis* Delvare & LaSalle (Hymenoptera: Eulophidae) is a polyphagous endoparasitoid of pupae of *Diatraea saccharalis* Fabr. (Lepidoptera: Pyralidae), *Anticarsia gemmatalis* Hüebner (Lepidoptera: Noctuidae), *Heliothis virescens* Fabr. (Lepidoptera: Noctuidae), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), *Thyrinteina arnobia* (Stoll) (Lepidoptera: Geometridae) (Bittencourt and Berti Filho 2004, Pereira et al. 2008) and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (Zanuncio et al. 2008). This ability of *P. elaeisis* to develop in different hosts may be related to immune suppression that occurs with these hosts after the oviposition of parasitoids possibly due to immunoregulatory substances present in the ovary of this parasitoid (Andrade et al. 2010b).

The female reproductive system of insects consists, in general, of a pair of ovaries, connected to a common oviduct by a pair of oviducts. Each ovary is composed by a series of ovarioles with a progressive development of oocytes, whose number is genetically determined and dependent on the species reproductive strategy (Klowden 2002). Each ovariole has two areas of oocyte maturation named germarium and vitellarium (Chapman 1998). The germarium contains stem line oogonias and prefollicular tissues. The oocytes are then produced by meiotic divisions surrounded by follicular cells involved in the yolk deposition and chorionogenesis (Chapman 1998). The deposition of nutritive substances in the oocyte is higher in the vitellarium (Klowden 2002, Lisboa et al. 2005).

Ovarioles of insects can be of panoistic or meroistic types (Davey 1985, Nijhout 1994, Chapman 1998). The first one is found in more primitive insects and the germarium usually contains only oogonias, primary oocytes and mesodermal prefollicular tissue. The meroistic ovary has structures named nurse cells (Klowden 2002). The ovaries can, also, be subdivided in teleotrophic and polytrophic types (Nijhout 1994, Chapman 1998).

The nurse cells of the meroistic teleotrophic ovariole are found in the apex of the ovariole and are responsible for nurturing the oocytes by the nutritive cord. The ovocytes form a group of cells interconnected to the nurse ones in the meroistic polytrophic ovariole (Chapman 1998).

The yolk accumulation occurs by two patterns. In the first one, the mature eggs are formed in the late larval or pupal stage and in the second the maturation of eggs occurs along the adult stage of the insect (Jervis et al. 2001).

The knowledge on the sexual maturation of female parasitoids is a prerequisite to improve the mass rearing of this natural enemy. Thus, the efficiency of the parasitism of *P. elaeisis* in pupae of *Bombyx mori* L. (Lepidoptera: Bombycidae) occurred at 72 and 96 h (Pereira et al. 2009). This may be related to the oogeny of this natural enemy. Thus, the aims of this paper were to study the oogenesis pattern and the type of ovariole of *P. elaeisis.*

MATERIALS AND METHODS

Palmistichus elaeisis and their alternative host (T. molitor pupae) were reared in the laboratory at 25 ± 1ºC, relative humidity of 70 ± 10% , and photophase: 14 h (Zanuncio et al. 2008).

Tenebrio molitor was reared on plastic trays (29 x 23 x 11 cm) with integral wheat flour (97%), beer yeast (3%) and slices of chayote as food and humidity source.

Four 72 h-old females of *P. elaeisis* received *T. molitor* pupae during 24 h in glass tubes (14.0 × 2.2 cm) covered with cotton and fed on honey (Zanuncio et al. 2008). Unmated females of this parasitoid were sexed by the morphologic characteristics of the antenna and abdomen (Delvare and LaSalle 1993) and individualized soon after their emergence from *T. molitor* pupae. The Zamboni fixative solution was utilized (Stefanini et al. 1967) to preserve newly-emerged females and at seven, 14, 24 and 48 h after their emergence.

The abdomens of six females of this insect were isolated per treatment, dehydrated in a graded ethanol series (70, 80, 90 and 95º) and embedded in a historesin (Leica). Twelve 4µm thickness sections per treatment were stained with hematoxyline and eosin and analyzed under light microscope. The mature eggs were characterized by the presence of chorion and absence of nurse cells (Chapman 1998).

RESULTS

The ovaries of all females of *P. elaeisis* under analysis were similar to those of other Hymenoptera with a pair of ovaries with four ovarioles/ovary. These ovarioles joined and formed a calyx that opens in the lateral oviducts (Fig. 1).

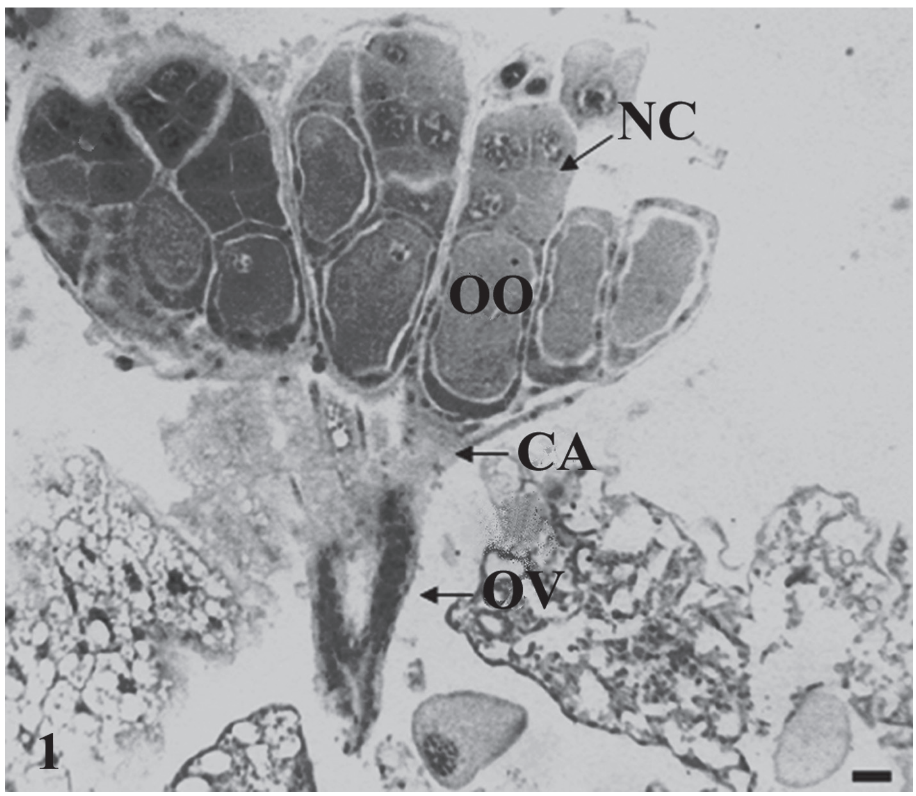

Figure 1 - Ovary of a newly-emerged females of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) from *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae: OO- oocyte, NC- nurse cell CA- calyx, OV- lateral oviduct. Bar: 10µm.

Palmistichus elaeisis presented an ovary of the meroistic polytrophic type due to its nurse cells being associated to the oocytes (Fig. 2). The nurse cells have well-developed nuclei (Fig. 3).

Newly-emerged females of *P. elaeisis* presented primary oocytes with the deposition of yolk and chorionogenesis, which was concluded 24 h after the female emergence (Fig. 4). Besides, the high quantity of eggs ready to oviposition occurred at 48 h-old females (Fig. 5). Females did not show matured eggs until 24 hours after emergence. The mean number of matured eggs by female was 22.00 ± 6.75 and 72.17 ± 14.23 in 24 and 48 h-old ones, respectively.

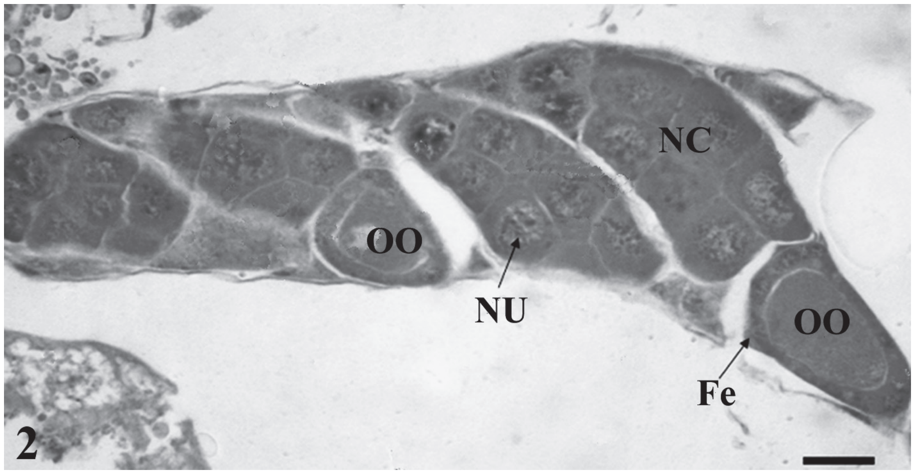

Figure 2 - Follicle (oocyte + nurse cells) of a newly-emerged females of *Palmistichus elaeisis* (Hymenoptera: Eulophidae). OO- oocyte, NU- nucleus of the nurse cells, NC- nurse cells, Fe– follicular epithelium. Bar: 10µm.

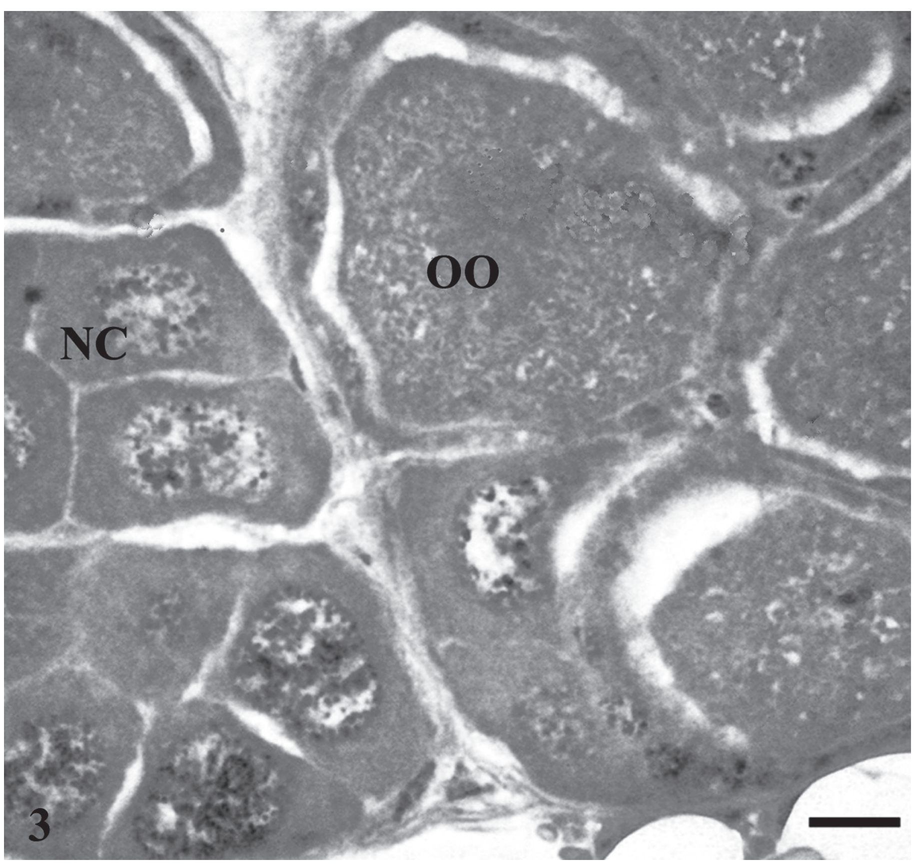

Figure 3 - Yolk accumulation in the oocytes (OO) of females of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) after seven hours of their emergence from *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae. NC – nurse cells. Bar: 10µm.

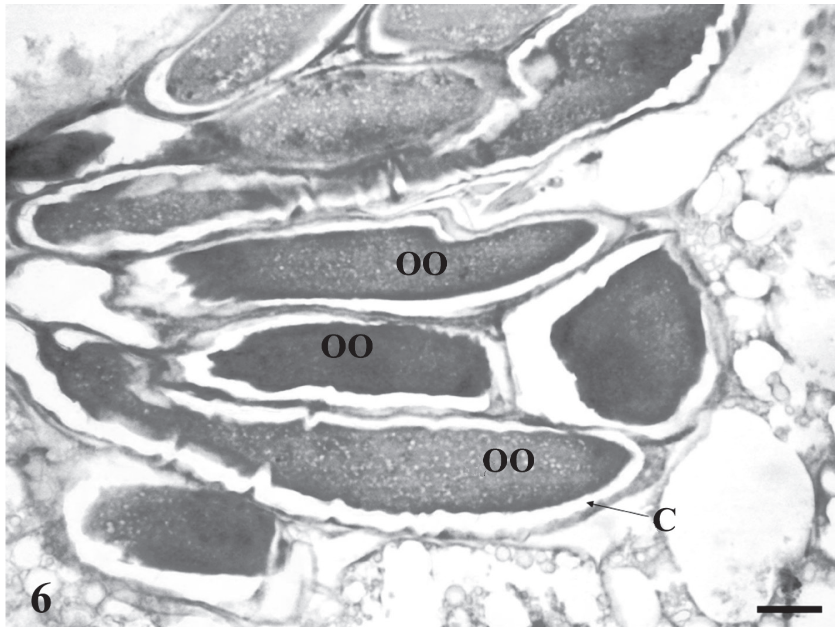

Figure 4 - Mature oocytes (OO) of females of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) 24 h after their emergence from *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae. **C**- chorion. Bar: 10μm.

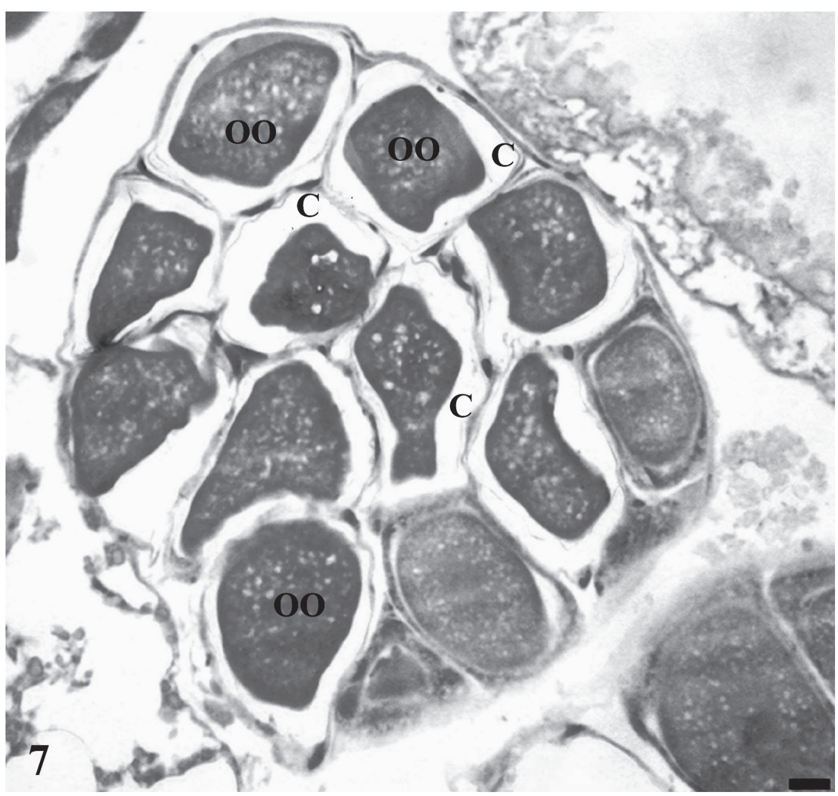

Figure 5 - Mature oocytes (OO) of females of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) 48 h after their emergence from *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae. **C**- chorion. Bar: 10μm.

DISCUSSION

The number of ovarioles per ovary is species-specific and varies from one (in some coleopterans) to more than thousands (in termites) (Nijhout 1994) depending on the size, life history and taxonomic position of the insect (Chapman 1998). However, intra-specific differences for the number of ovarioles in insects can occur due to genetic and stressing factors as starvation, and the largest number of ovarioles has no positive correlation with the fecundity of *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) (Wayne et al. 2006). The oogenesis of females of *P. elaeisis*, as demonstrated after the emergence, is similar to that of reports on egg maturation of many Hymenoptera species (Jervis et al. 2001, Martins and Serrão 2004). The gradual oocyte growth associated with the increase of *P. elaesis* age is related to the yolk accumulation of this insect with a pattern similar to most Hymenoptera species (Jervis et al. 2001). The number of mature eggs in newly-emerged parasitoids allows them to produce a great number of eggs/oviposition, but the reduction of the host availability decreases the parasitism rate (Jervis et al. 2001, Jervis and Ferns 2004). On the other hand, the low proportion of mature eggs is a disadvantage because only a small fraction of the life period of parasitoids is used for egg production, but it gives a larger reproductive plasticity for variations on host availability (Ellers and Jervis 2003, Jervis and Ferns 2004). Synovigenic species have yolk-rich eggs. This suggests the presence of a great amount of fat body, low ovigeny index, relatively long life time, gradual increase until the stabilization of the oviposition and feeding behavior of *P. elaeisis* (Jervis et al. 2001).

The gradual development of the oocytes after their emergence in insects was associated with a high number of fat body cells, which are the main cells responsible for the vitellogenin synthesis (Palli and Locke 2008), which is the case of females of *P. elaeisis*. Females of *P. elaeisis* starved for 72 h present high parasitism. This indicates that the metabolic reserves may be used for egg production without compromising their development during the period evaluated (Ueno and Ueno 2007, Ozkan 2007). However, the maturation by endogenous regulation can be retarded when the host has low nutrient quality or quantity or when it offers that they offer low energy for the parasitism of synovigenic insects (Desouhant et al. 2005, Wu and Heimpel 2007). On the other hand, food sources cannot influence the egg maturation of parasitoids (Sisterson and Averill 2002).

The complete development of oocytes of *P. elaeisis* was observed in 24 h-old females and, as for other species, the yolk deposition in the oocytes occurred in the proximal area of the ovariole (Klowden 2002). The death of follicular cells after the complete synthesis of the chorion results in the inhibition of the transport of substances to the oocyte (Klowden 2002, Martins and Serrão 2004).

One possible response to the higher reproductive success of *P. elaesis* aged between 72 and 96 h compared with younger individuals in *B. mori* (Pereira et al. 2009) is the maturation of oocytes occurring throughout the adult life of the parasitoid with a high concentration of eggs after 48 h, as demonstrated in this study. Thus, these findings may help to improve the mass production of *P. elaeisis*, understand some characteristics of the reproductive physiology of Eulophidae and predict the release time to improve the efficiency of *P. elaeisis*.

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RESUMO

O conhecimento da ovigenia em parasitóides é importante para estudos básicos em fisiologia e para o controle biológico aplicado. O padrão de ovigenia e o tipo de ovaríolo do parasitóide *Palmistichus elaeisis* Delvare & LaSalle (Hymenopera: Eulophidae) foram estudados em fêmeas recém-emergidas e em intervalos de sete, 14, 24 de 48 horas após a emergência em pupas de *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). Fêmeas de *P. elaesis* apresentaram o ovário composto por quatro ovaríolos do tipo meroístico politrófico. A deposição de vitelo e corionogênese em *P. elaeisis* foram concluídas 24 horas após a emergência. Fêmeas com 48 horas de idade apresentam grande quantidade de ovos prontos para a oviposição. Esses resultados podem ajudar a melhoria da produção massal de *P. elaeisis* e o controle biológico aplicado com esse parasitóide.

Palavras-chave: Controle biológico, morfologia, inimigo natural, ovigenia.

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