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REVIEW

Oenocytes in insects

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Abstract

Oenocytes are insect cells responsible for lipid processing and detoxification. Of ectodermic origin, they are found in close association with the insect epidermis, or fat body cells, or both depending on the insect species and developmental stage. They are easily distinguishable either by staining or by their ability to form cell clusters lined by a basal lamina, which makes it possible to isolate them from other cells. The most noticeable characteristic of the oenocytes ultrastructure is the presence of a well-developed smooth endoplasmic reticulum that can fill almost entire cell cytoplasm that for a long time was suggestive of lipid processing capacity. This capacity was confirmed lately through the usage of genetic, molecular and biochemistry approaches and other functions are also addressed to these cells, such as cuticular hydrocarbons and pheromones synthesis and detoxification. Additionally, oenocytes are considered analogous to mammalian hepatocytes based on their gene expression profiles and cell functions. In spite of the current knowledge about oenocytes, much about their protein expression profile remains unknown. In this review we provide a general overview of the state of the art related to oenocytes studies and certain morphological and biochemical aspects of such cells crucial for insect survival.

Key Words: insects; oenocytes; oenocyte ultrastructure; oenocyte metabolism

Introduction

Oenocytes are polyploid insect cells of ectodermic origin and are usually found in close association with the epidermis or fat body cells, or both depending on the insect species and developmental stages (Locke, 1969; Dorn and Romer, 1976; Hartenstein *et al.*, 1992). Although oenocytes have been known for over a century (Landois, 1865; Koschevnikov, 1900; Imms, 1907; Vickery, 1915) only recently their role in lipid metabolism and detoxification has been confirmed. According to Snodgrass (1935) the term oenocyte refers to the usual pale amber color of the cells (oeno or oinos means wine in Greek), however oenocytes may also display colorations that vary from brown, to yellow, to green, or red, and sometimes are even colorless. Koschevnikov (1900) was one of the first to speculate on the possible role played by oenocytes (wrongly thought to be "urinary cells"), however, until the early 2000's oenocytes were considered one of the least studied cells of invertebrates (Gould *et al.,* 2001). More recently, aspects of oenocytes differentiation (Elstob *et al.*, 2001; Burns *et al.*, 2012), gene expression (Lycett *et al.*, 2006; Gutierrez *et al.*, 2007; Martins *et al.,* 2011a), biochemistry (Wicker-Thomas *et al.*, 2009) and physiological roles (Knauf *et al.*, 2002; Gutierrez *et al.*, 2007) have been investigated in details.

Arthropods utilize different strategies against the harmful effects of waste metabolites. Such strategies may include metabolites excretion by feces and urine, and also neutralizing and storing them in the fat body. Lycett *et al.* (2006) demonstrated that oenocytes play an important role in detoxification, protecting the organism against toxic and potentially lethal compounds such as insecticides. Further, through the synthesis of hydrocarbons present on the outer surface of the insect cuticle (reviewed by Lockey, 1988), oenocytes have a role in preventing water loss (Fan *et al.*, 2003), and have been shown to participate in the intraspecific communication as chemical signals (Wicker-Thomas *et al.*, 2009).

Oenocytes are crucial for insect survival as indicated by their many key roles. Here we provide

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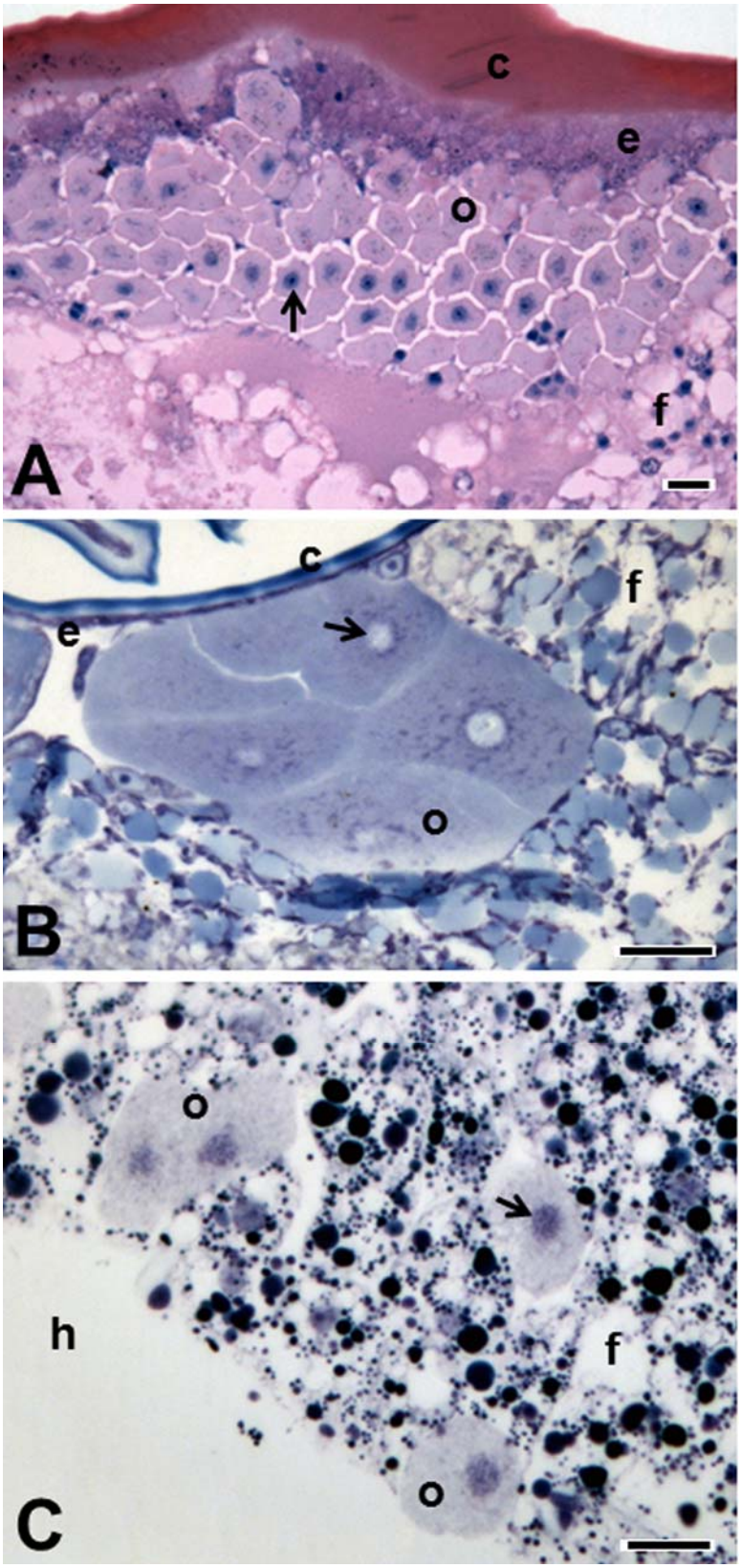

Fig. 1 Histological sections showing the location and organization of oenocytes in insects. Hematoxylin and Eosin staining of oenocytes (o) located underneath the abdominal epidermis (e) in *Brontocoris tabidus* (Heteroptera; Pentatomidae) (5^{th} instar nymph) is shown in **A**. Toluidine Blue staining of oenocytes (o) of *Toxorhynchites theobaldi* (Diptera; Culicidae) 4^{th} larva (**B**), where clustered oenocytes are attached to the epidermis (e) and in close association with the fat body (f); and newly-emerged adult female (**C**), where oenocytes are seen as clustered or single cells scattered between trophocytes in the periphery or inside the fat body (f). Arrow- oenocyte nucleus; c- cuticle; h- hemolymph. Bar = 10 µm.

a comprehensive review on the roles played by oenocytes in insects, and details of these cells' location, general morphology, ultrastructure, biochemistry and gene expression.

Oenocytes location and types

The location of the oenocytes within insect body varies depending on the species or developmental stage. They can be found either associated with the epidermis (Figs 1A-B), scattered between abdominal fat body cells (Fig. 1C), or both within the same individual, as summarized in Table 1.

When associated with the abdominal fat body cells, oenocytes are organized as either single or clustered cells within the same insect. In *Aedes aegypti* (Culicidae) larvae, they are associated with the epidermis and located among the parietal fat body cells (Wigglesworth, 1942). This also is the case for *Toxorhynchites theobaldi* (Culicidae) larvae (Martins *et al.*, unpublished data) (Fig. 1B). In adults, however, the oenocytes appear in the periphery of the fat body, or inside fat body lobes either as single or clustered cells in a manner similar to what is observed in other haematophagous mosquitoes (Martins *et al.*, 2011b, c) (Fig. 1C). According to Fan *et al.* (2003), oenocytes that are arranged in discrete clusters within the hemocel are readily accessible for experimentation, and most investigations have concentrated on insect species whose oenocytes display such arrangement. Moreover, the presence of oenocytes cell clusters facilitates their dissection and the establishment of *in vitro* culture (Krupp and Levine 2010; Martins *et al.,* 2011a, d).

Previously it has also been shown that in larvae of the fruit fly *Drosophila melanogaster*, oenocytes form groups of four to nine cells attached to the lateral epidermis of each body segment. In addition, *D. melanogaster* oenocytes do not divide following eclosion of the larvae and simply grow in size (Reviewed by Elstob *et al.*, 2001; Gould *et al.*, 2001).

In larvae of *Anopheles maculipennis* (Culicidae) oenocytes are separated in large and small. Large oenocytes are segmentally arranged in clusters, and are present in each of the first seven abdominal segments. They are absent from abdominal segments eight and nine, and from the thorax. In each of the larval abdominal segments of *A. maculipennis* oenocytes are located ventro-laterally and consist of two pairs of vary large cells on both sides of abdomen. Small oenocytes on the other hand are numerous and have no defined arrangement although they are sometimes found in pairs.The small oenocytes are present just beneath the epidermis in the vicinity of each of the clusters formed by the large oenocytes. They are located anteriorly with respect to the large oenocytes but can be found along the base of each segment on either side of the nerve cord. Small oenocytes are generally observed in the first eight abdominal segments and occasionally in the last segment (Imms, 1907).

In adults of *Apis mellifera* (Apidae), different populations of oenocytes also can be distinguished according to their localization and size. In newly-emerged queens, the small oenocytes are parietal and present in small groups immersed in the fat body while larger oenocytes appear as isolated cells scattered in the perivisceral fat body. In older queens only one population of oenocytes is present in the parietal fat body, and located mainly ventrally (Hepburn *et al.*, 1991). For the worker bees, the number of oenocytes varies according to age and body segment, with younger individuals having fewer oenocytes than the older workers, and there are fewer oenocytes in their head as compared to the abdomen. In addition, head oenocytes are larger than those in the abdomen, with bigger cellular and nuclear volumes (Ruvolo and Cruz-Landim, 1993).

The association between fat body cells and oenocytes persists throughout all developmental stages of the locust *Schistocerca gregaria*. In this species, oenocytes are more numerous in the subepidermal region, and are intimately associated with trophocytes (Coupland, 1975).

In the beetles *Tenebrio molitor* and *Tenebrio obscurus*, adult insects display oenocytes arranged in grape-like clusters along the length of the dorsal surface of the lateral longitudinal trachea of the abdomen, but may also extend upwards along the dorsal trachea in the region of the spiracles. These clusters, made of a variable number of individual cells and with diameters ranging from 96 to 170 µm, lie within among the fat body and can be easily recognized by their brown color (Roth, 1942).

In nymphs and adults of heteropterans, oenocytes are located between the basal membrane and the epidermis and are clearly distinct from the fat body cells, as observed in the soldier bug *Brontocoris tabidus* (Pentatomidae) nymph (Martins *et al.*, unpublished data) (Fig. 1A). In kissing bugs, such as *Rhodnius prolixus* and *Triatoma infestans* (Triatominae), oenocytes are located in tergites and sternites in the abdomen, with sizes ranging from 10 to 100 µm, depending on the developmental stage [Juárez and Fernández (2007) and references therein]. Wigglesworth (1933) observed that at the time of blood-feeding of *R. prolixus* nymphs there are two types of oenocytes: one large solitary and more or less lobulated with condensed chromatin near the center of the nucleus, and another small rounded oenocyte considered to be a new generation of cells. These newly generated cells can be differentiated as they are almost always in pairs and are sometimes linked by an unbroken strand of cytoplasm with the condensed chromatin more evenly distributed over the nuclear membrane.

In adult *A. aegypti*, oenocytes are located on the periphery of the fat body and between trophocytes of the abdominal fat body. Each rounded or oval cell is surrounded by a thin, finely granular basal lamina (Tadkowski *et al.*, 1977; Martins *et al.*, 2011b). Similarly, in the stingless bee *Melipona quadrifasciata* numerous oenocytes occur among the fat body cells and they have small nucleus and an acidophilic cytoplasm (Paes-de-Oliveira and Cruz-Landim, 2006).

The greatest polymorphism observed in insect oenocytes has been described in *Calpodes etlhius* (Lepidoptera), and that also seem to be the case for

Table 1 Distribution and location of oenocytes according to insect species, order and developmental stage

Insect species	Order (common name)	Stage	Oenocytes location and organization	Reference
Monomorium pharaonis	Hymenoptera (pharaoh ant)		associated to abdominal trophocytes in the parietal fat body	Jensen and Børgesen, 2000
Cyphomyrmex rimosus *Mycetarotes parallelus* *Acromyrmex disciger* *Atta laevigata*	Hymenoptera (leaf-cutting ants)		associated to trophocytes in the parietal and perivisceral fat bodies	Roma *et al.,* 2006, 2008
Pachycondyla villosa	Hymenoptera (panther ants)	adult	distributed among the trophocytes; right underneath the epidermis; isolated or in cluster of three to five cells, in both the thorax and abdomen, and more abundant in the latter, near the intersegmentary muscles	Zara and Caetano, 2004
Pachycondyla striata			distributed among the trophocytes	Thiele and Camargo-Mathias, 2003
Apis mellifera	Hymenoptera (honey bee)	adult	below epidermis in close association with the fat bodies	Hepburn *et al.,* 1991; Ruvolo and Cruz-Landim, 1993
Musca domestica	Diptera (house fly)	larvae	near the periphery of the abdominal segments and attached singly or in groups to the tracheae	Studinger and Willig, 1975
Dacus tryoni	Diptera (Queensland fruit fly)		are located between the epidermis and the fat body	Evans, 1967
Drosophila melanogaster	Diptera (fruit fly)	larvae	arranged in lateral and subepidermal clusters of, on average, 6 cells per abdominal hemisegment	Elstob *et al.*, 2001; Gutierrez *et al.*, 2007
		adult	subcuticular abdominal cells found in segmentally repeated rows that form crescent-shaped strands on the tergites and small clusters on the sternites	Ferveur *et al.*, 1997; Billeter *et al.*, 2009
Aedes aegypti	Diptera (yelow fever mosquito)	adult pupa		Martins *et al.,* 2011b, c
		larva	clustered or single cells scattered between trophocytes in the parietal fat body	Wigglesworth, 1942
Aedes albopictus, *Aedes fluviatilis* *Culex quinquefasciatus* *Anopheles aquasalis* *Anopheles darlingi*	Diptera (hematophagous mosquitos)	adult		Martins *et al.,* 2011c
Anopheles maculipennis		larvae	located ventro-laterally, consisting of two pairs of large cells on both sides of abdomen	Imms, 1907
Toxorhynchites theobaldi	Diptera (mosquito hawk)	larva	clustered in the parietal fat body	Martins *et al.*, unpublished
		adult	clustered or single cells scattered between trophocytes in the parietal fat body	

Brontocoris tabidus	Heteroptera (soldier bug)	fourth instar nymph		
Rhodnius prolixus *Triatoma infestans*	Heteroptera (kissing bugs)	nymph and adult	attached to epidermis	Juárez and Fernández, 2007; Wigglesworth, 1933
Oncopeltus fasciatus	Heteroptera (milkweed bug)	embryo	spread between trophocytes	Dorn and Romer, 1976
Echidnophaga oshanini	Aphaniptera (flea)	adult	associated to trophocytes in the parietal fat body	Vashchenok, 1966
Blattella germanica	Blattaria (German cockroach)		located beneath the epidermis, close to the basal lamina	Fan *et al.*, 2003
Leucophaea maderae	Blattaria (Madeira cockroach)	embryo	included in the epidermis, associated to dermal glandular cells	Rinterknecht, 1985
Lipeurus lawrensis tropicalis	Phthiraptera (lice)	nymph and adult	attached to epidermis, single or in cluster of two-six cells	Saxena and Agarwal, 1980
Schistocerca gregaria	Orthoptera (desert locust)	adult	in close association with the fat body	Coupland, 1975
		nymph	parietal abdominal fat body in association with trophocytes and urate cells	Diehl, 1973
Bombyx mori	Lepidoptera (silkworm)	larvae	metamerical groups on both sides of the abdomen associated with the spiracles	Vickery, 1915
Calpodes ethlius	Lepidoptera (Brazilian skipper)		close to specialized wax glands and subepidermal	Locke, 1969
Tenebrio molitor *Tenebrioobscuros*	Coleoptera (darkling beetles)	adult	in close association with the fat bodies and tracheal system	Roth, 1942
Attagenus megatoma	Coleoptera (black carpet beetle)	larva and adult	in each of abdominal segments and in the secondand third adult thoracic segments; in cluster of five to eight cells associated with dorsoventral muscles toward the anterior of each ofthese segments; often associated with fat bodies and trachea	Dunkel and Mallory, 1968

other hesperids. Also, three types of oenocytes can be recognized in the larvae according to location and morphology (Locke, 1969): permanent oenocytes, which are located below the wax glands and remain enlarged throughout the intermoult period; segmentally arranged oenocytes, which only enlarge during the moulting cycle; and smaller subepidermal oenocytes, which appear just before pupation and occur in large numbers. Although the role(s) of these three types of oenocytes is yet to be determined, they are likely to be distinct (Locke, 1969).

Oenocytes in other arthropods

In addition to insects, oenocytes have been described in Chelicerata (Romer and Gnatzy, 1981), Miriapoda (Fontanetti *et al.*, 2004), and Crustacea (Symonová and Smrž, 2009). However, these reports are few and mostly descriptive.

In adult opilionid (Arachnida), oenocytes were described in the legs (Romer and Gnatzy, 1981), wedged between the bases of the epidermal cells and their cytoplasm. Like their counterparts in insects, Arachnida oenocytes also are

distinguishable by the presence of well-developed smooth endoplasmic reticulum (SER) (see below). Another notable characteristic is the presence of many autophagic vacuoles and the conspicuous infoldings of the basal plasma membrane (Romer and Gnatzy, 1981). Oenocytes were reported associated with the fat body cells of *Rhinocricus padbergi* (Diplopoda, Spirobolida) adults (Camargo-Mathias and Fontanetti, 2000; Fontanetti *et al.*, 2004; Souza *et al.*, 2011) and they are very similar to the insect oenocytes in terms of location, staining properties and ultrastructure that will be discussed further.

In Crustacea, oenocytes were described in juveniles and adults of ostracodes where they are present in large numbers in the body cavity and in the appendages of juveniles but less frequent in adults. Their shape has been described as irregularly oval, measuring approximately two-three µm in diameter (Symonová and Smrž, 2009).

Oenocytes changes during post-embryonic development

Oenocytes arise during embryonic and post-embryonic development and the differentiation of their ectodermic precursors have been studied in several insect species (Lawrence and Johnston, 1982; Rinterknecht and Matz, 1983; Hartenstein *et al.*, 1992; Burns *et al.*, 2012). Oenocytes formation and differentiation also occur during insect post-embryonic development, however, it is restricted to metamorphosis and ecdysis in holo- and hemimetabolous insects, respectively (Wigglesworth, 1933; Evans, 1967).

During metamorphosis, oenocytes have been shown to arise via *de novo* mechanisms. In *Dacus tryoni* (Diptera, Trypetidae), larval oenocytes dissociate and eventually disintegrate simultaneously with the larval fat body cells during metamorphosis. At the beginning of pupation, segmentally arranged clusters of small cells lie among the large cells of the larval epidermis. Between the second and fourth days following pupation, the smaller cells invade the surrounding epidermis, displacing it, and forming the adult epidermis. In this process, some of the adult cells migrate from the clusters into the body cavity (becoming free cells). By the sixth day of the pupal stage, these free cells have increased in size and are observed scattered under the entire surface of the abdominal integument. In addition, at this point the free cells become multinucleated and during the next few days will continue to enlarge. From the time they first appear, the adult oenocytes gradually increase in size until emergence of the adult insect (Evans, 1967).

It has been shown that oenocytes obtained from the last instar larvae of the cabbage armyworm *Mamestra brassicae* (Lepidoptera) can differentiate *in vitro*. Moreover, they also are capable of affecting differentiation of the wing imaginal discs when in culture in the presence of the prothoracic gland and in media containing oenocytes and α-ecdysone. However, the wing discs display greater development than when cultured with α-ecdysone alone (Agui, 1974).

Table 2 Oenocyte size, number, and proportion according to abdominal volume in *Drosophila melanogaster* (Johson and Butterworth, 1985).

Age (days)	Oenocytes number	Oenocyte Size (μm^2)	Oenocytes as a percentage of abdominal volume (%)
Female			
0-1	7,138	315	1.7
6-7	5,855	475	1.4
56	4,819	658	1.5
Male			
0-1	4,224	257	1.1
6-7	6,602	715	3.2
56	5,271	665	3.3

Morphometric analyses showed that in *D. melanogaster* the size and number of oenocytes vary greatly during aging of the adult insect and according to sex (Johnson and Butterworth, 1985) (Table 2). Interestingly, for the aging *D. melanogaster* male, an increase in cell size (nearly three times the size observed in newly emerged) translates in the oenocytes representing almost three percent of the abdomen of the fruit fly (Table 2). Morphologic and morphometric aspects of *A. mellifera* oenocytes were also studied in queens and workers in different adult ages (Ruvolo and Cruz-Landim, 1993, 1995). For instance, size increase of 10.18 % and 7.47 % were noticed in *A. mellifera* workers oenocytesin the 5^{th} and 7^{th} days post emergence, respectively (Ruvolo and Cruz-Landim, 1995).

After *R. prolixus* nymph blood-feeding, whereas the small oenocytes grow rapidly, acquiring pseudopodia, old large cells break down completely. Nine days post blood meal the oenocytes gradually reduce their size, and this trend continues for a few days after the next moulting as the new cuticle is being formed. Oenocytes do not change until the next blood meal followed by a new moulting cycle. Thus, in *R. prolixus* a new generation of oenocytes arises at each moulting cycle from undifferentiated cells in the epidermis with the oenocytes from the previous instar persisting into this new stage of development (Wigglesworth, 1933).

Oenocytes hystochemistryand cytochemistry

One of the main features used to identify oenocytes in histological sections is their acidophilic cytoplasm by using routine laboratory staining such as Hematoxylin and Eosin and Toluidine Blue (Figs 1A-C). Additionally, different cell staining methods including the Bromophenol Blue and Millon Reaction for proteins, Periodic Acid Schiff (PAS) for neutral sugars, Feulgen Reaction for DNA, Sudan Black (SB), Oil-Red and Osmium (OsO_4) impregnation for lipids and Pyronin for RNA (Locke, 1969; Coupland, 1975; Zara and Caetano, 2004; Roma *et al.*, 2006, Gutierrez *et al.*, 2007; Roma *et al.*, 2008; Martins *et al.*, 2011b) have been extensively applied to the

study of oenocytes in several insects, helping to reveal their distinguishable staining properties. These properties include the oenocytes' cytoplasm positivity for proteins and lipids, and non-positivity for polysaccharides as discussed below. In addition, streptavidin also has been used to help track the fate of oenocytes during embryonic development and the differentiation of its ectodermic precursor in in the red floor beetle *Tribolium castaneum* (Burns *et al.*, 2012).

In oenocytes of adult eusocial ant workers such as the basal Attini *Cyphomyrmex rimosus* and *Mycetarotes parallelus* and the derived *Acromyrmex disciger* and *Atta laevigata* strong reaction to Bromophenol Blue indicated the presence of large amounts of proteins in these cells (Zara and Caetano, 2004; Roma *et al.*, 2006). Oenocytes of the parietal and perivisceral fat bodies of *C. rimosus* and *M. parallelus* are characterized by small and very electrondense protein granules distributed throughout their cytoplasm. On the other hand, lipids with large electrondense granules are commonly found and basic proteins are represented by granules in oenocytes of all species (Roma *et al.,* 2008). These studies also demonstrated that oenocytes are weakly positive for the PAS test (Zara and Caetano, 2004; Roma *et al.*, 2006, 2008). Histochemical tests for detection of lipids also evidenced the presence of positive cytoplasmic inclusions that contain unsaturated lipids that are more abundant in oenocytes of derived ants than basal ones (Roma *et al.*, 2008). Additional details of oenocytes organization and staining profile in ants can be found elsewhere (Roma *et al.,* 2010).

In *T. molitor* larvae, oenocytes impregnated with OsO_4 displayed different levels of lipid staining, which was also reported for the staining of esterases with naphthyl-acetate. In addition, oenocytes in *T. molitor* pupal and adult stages contain lipases as demonstrated by the presence of brownish crystals as the end product of this enzyme activity (leadsulfide) (Romer, 1980).

In the adult honeybee *A. mellifera* Ruvolo and Cruz-Landim (1993) showed that the staining pattern of oenocytes varied according to the caste. For example, the abdominal oenocytes of queens are less acidophilic and showed weak reaction to SB than those of workers. These authors pointed that the differences between the oenocytes of queens and workers indicate that these cells may have different functions in the two castes. In 12-day old bees oenocytes showed a very strong reaction to SB and the intensity of staining is reduced gradually between 17- and 29-day old bees. Treatment with Nile Blue also indicated that these cells contain acid lipids that could be participating in wax synthesis.

Acid phosphatase (AP) was also observed in oenocytes in all workers and queens of *A. mellifera* adults, with queens displaying comparatively larger amounts of the enzyme. Moreover, in workers, the levels of AP are higher in five- and 29-day old bees. For queens, the presence of AP within oenocytes throughout their life is consistent with continued intracellular digestion activity in oenocytes, as AP is frequently associated with lysosomes (Ruvolo and Cruz-Landim, 1993).

Worker honeybees are sensitive to Earth's magnetic field. In a provocative study, Kuterbach *et al.* (1982) indicated that oenocytes in this caste of adult bees contain magnetite (Fe_3O_4) as numerous electron-opaque, iron containing granules in the cytoplasm and are concentrated in the ventral abdomen under each segmental ganglion in the adult foraging worker. In light of the high iron content of these cells, these investigators suggested that in honey bees these oenocytes are involved in insect orientation.

Our studies on oenocytes of *A. aegypti* adult females indicated that, histochemically, these cells display a PAS-negative cytoplasm, which also was strongly and uniformly stained by Bromophenol Blue and OsO_4 (Martins *et al.*, 2011b). However, the staining intensity changed depending on the diet. For example, in blood-fed mosquitoes the oenocytes are weakly stained in comparison to newly-emerged and sugar-fed individuals. Oenocytes are positive for lipid staining and negative for PAS reaction indicating that polysaccharides content is not as pronounced as the amount of proteins and lipids. Similar results also were found for several mosquito species including *Aedes albopictus*, *Aedes fluviatilis*, *Culex quinquefasciatus*, *Anopheles aquasalis* and *Anopheles darlingi*. In these insects the oenocytes also have uniformly stained cytoplasm (using Gomori's Trichrome) confirming the high protein content in these cells (Martins *et al.*, 2011c).

The cytoplasm of oenocytes has also been shown to be poor of glycogen in several insects. This is the case in *A. aegypti* (Tadkowski *et al.*, 1977*;* Martins *et al.*, 2011b) and in at least four ant species (Zara and Caetano 2004; Roma *et al.,* 2006, 2008). For the cricket *Gryllus bimaculatus* (Orthoptera) no glycogen is observed within the first 25 h of the first instar (Romer, 1974). However, glycogen can be detected as PAS-positive spots reaching nearly 3 µm in diameter within the cells of the cricket as larvae development proceeds. In crickets shortly before moulting glycogen rosettes have completely disappeared. These observations suggest that glycogen function as energy source for the increasing cell metabolism towards the end of the *G. bimaculatus* first instar (Romer, 1974). It seems that the presence of large amount of glycogen is not common in insect oenocytes and generally is restricted to early developmental stages as an energy supply. In agreement with these observations, following apolysis of *L. maderae* oenocytes still display clusters of glycogen that remain until soon after the first larval epicuticle deposition (Rinterknecht, 1985). An exception to the availability of glycogen in insect oenocytes described above is *Dacus tryoni* (Diptera, Trypetidae). Oenocytes in adults of this species display many electron-dense granules, likely glycogen, throughout their cytoplasm (Evans, 1967).

Regarding ribosomes, Romer (1974) used Pyronine staining during the molting cycle of *G. bimaculatus* nymphs to assess oenocytes profile. Under light microscopy, oenocytes display a patchy staining and under the transmission electron microscope (TEM) few ribosomes are found in oenocytes from immediately post-hatch larvae and

this condition changes 12 h post moulting. Especially around the nucleus, many extended polysomes accumulate ribosomes and the number of ribosomes becomes maximal mainly in the regions of rough endoplasmic reticulum (RER) 35 to 55 h post moult in *G. bimaculatus*.

Oenocytes ultrastructure

One of the most striking albeit not surprising characteristics of insect oenocytes is the presence of a well-developed SER. The oenocyte SER sometimes occupies almost all of the cell's cytoplasm (Martins *et al.,* 2011d), resembling steroidogenic cells (Rinterknecht and Matz, 1983), and this is in accordance with their function in lipid synthesis and processing and detoxification (Evans, 1967; Locke, 1969; Clark and Dahm, 1973; Martins *et al.,* 2011b). The oenocyte's SER consists of a net of ramified tubules that in the case of *G. bimaculatus* nymphs can vary in size from 170 to 340 Å depending on age (Romer, 1974).

The development of such prominent SER may start during the insect's early developmental stages (i.e. embryo) (Dorn and Romer, 1976; Rinterknecht and Matz, 1983) and can also occur during the entire larval, pupal and adult stages (Locke, 1969; Romer, 1974; Stoppie *et al.,* 1981; Martins *et al.*, 2011b, d). SER-associated organelles such as the multivesicular bodies, the peroxisome-like organelles, and the microbodies described in the insect oenocytes are discussed below. In comparison to the SER, the RER and Golgi complex are not prominent in oenocytes. In general, the RER is formed by short stacks that are restricted to certain areas of the cytoplasm (Wigglesworth, 1933) or limited to the perinuclear region (Tadkowski *et al.,* 1977; Martins *et al.,* 2011b, d). These subcellular characteristics are not only observed in adult oenoyctes but are also seen in oenocytes during early insect embryogenesis (Dorn and Romer, 1976).

Changes in the ultrastructure of oenocytes were studied considering embryonic (Dorn and Romer, 1976; Rinterknecht, 1985) and post-embryonic developments, including hemi- and holometabolous insects (Wigglesworth, 1933; Evans, 1967; Locke, 1969; Romer, 1974; Dorn and Romer, 1976). In general, oenocytes are always separated from the fat body cells and other tissues by a basal lamina (Stoppie *et al.,* 1981) and another notable characteristic is the abundant number of mitochondria scattered throughout their cytoplasm (Romer, 1974; Clark and Dahm, 1973; Sohal, 1973).

During the early embryogenesis the oenocyte cell population is heterogeneous. Rinterknecht and Matz (1983) reported an entire scale of intermediate levels of differentiation in *L. maderae*. However, it has not been possible to establish whether this heterogeneity reflects the existence of a plurality of cell functions, or whether this is related to the progress in terms of differentiation of a unique cell population. When individual pleuropodial cuticle deposition occurs there is a rapid increase in the number of differentiating oenocytes of *L. maderae* embryo. At the time of dorsal closure, the deposition of the epicuticle of the embryonic cuticle is preceded by the SER proliferation and by the differentiation of a new oenocyte generation (Rinterknecht and Matz, 1983). Just before general apolysis and during its occurence, the oenocytes display the well-developed SER. The oenocyte population then regresses after epicuticle deposition of the first larval cuticle. Just prior to the cuticulin layer of the embryonic cuticle is observed, another wave of oenocyte differentiation takes place. In this case, oenocyte differentiation is marked by a rapid biogenesis of the cell membrane and was correlated with the ectodermal coating, the titer of the ecdysone and the differentiation of the prothoracic gland (Rinterknecht, 1985).

In *Musca domestica* (Diptera) oenocytes change according to age in adults and in contrast to other insect which display mononucleated oenocytes, here oenocytes either have one or two nuclei (Clark and Dahm, 1973; Sohal, 1973). Curiously, no difference in the appearance of oenocytes between adult males or females has been described for *M. domestica* (Clark and Dahm, 1973). Differently to four-day-old flies, in older flies (over 30 days), oenocytes undergo a variety of degenerative alterations that may include reduction in the quantity of elements of the SER, a reduction in the matricial density and the number of cristae in mitochondria and the vacuolization of several areas of the cytoplasm. Another prominent feature of the degenerating oenocytes of *M. domestica* adults is the presence of lysosomes (Sohal, 1973).

In *Glossina austeni* (Diptera) adult females, oenocytes exhibit cyclical changes during the post-emergence growth and during subsequent cycles of pregnancy. In newly emerged flies, the cytoplasm of the oenocytes contains a few deeply staining inclusions, which become more prominent during the period between emergence and ovulation and remain large during the first cycle of pregnancy (Tobe *et al.,* 1973). In one-two-day-old *Sarcophaga bullata* (Diptera) adults, the SER is already very dense with many small vesicles. During vitellogenesis of this species, the oenocytes display invaginations of the plasma membrane and high electron dense granules in their cytoplasm that accumulate following completion of vitellogenesis. In the post-vitellogenesis period, the mitochondria become more spherical and the typical parallel arrangement of the cristae disappears. In spite of all the changes observed, it is still not clear what role oenocytes play during the process of adult vitellogenesis of *S. bullata* (Stoppie *et al.,* 1981).

Oenocytes of the *O. fasciatus* embryo, which has just differentiated, have a basal membrane already formed and the mitochondria are relatively large, but not as numerous. After migration of the oenocytes into the fat body, pronounced changes in cellular structure occur with the SER occupying almost the whole cytoplasm. Vacuoles only occur in embryos and adults, not in larvae, however, microbodies are numerous in the fifth larvae. Here, anchorage of oenocytes to fat body cells by desmosomes is occasionally seen in the embryo, but not in larvae or adults of *O. fasciatus* (Dorn and Romer, 1976).

In *A. aegypti* adults, oenocytes have a single, large, round, centrally-located nucleus with a

prominent nucleolus and chromatin appears in irregular granular clumps, especially around the edge of the nucleus (Tadkowski *et al.,* 1977; Martins *et al.,* 2011b, d). Yet in the mosquito, the oenocytes ultrastructure changes significantly form pupae to adults. In pupae the SER occupies the large extensions of the cytoplasm, while in adults SER are restricted to some areas (for details see Martins *et al.,* 2011b, d).

A close correlation is also to be observed between the moulting cycle and the differentiation of Golgi complex in oenocytes of *G. bimaculatus* nymphs. Immediately after hatching, several Golgi complexes are found and certain areas show coated vesicles. As RER increases, the number of Golgi complex decreases, but increases again close to the end of the moult. Some mitochondria show a circular arrangement of the cristae and they appear in cells showing pronounced lytic activity close to moulting (Romer, 1974).

Fifty four hours after molting of *G. bimaculatus*, oenocytes cytoplasmic membrane has infoldings, reaching almost to the nucleus (Romer, 1974). These canaliculli are observed around the cell's periphery in *A. aegypti*, sometimes with delicate membranous and/or fine amorphous flocculent material (Tadkowski *et al.,* 1977; Martins *et al.,* 2011b, d). They are more developed in *A. aegypti* blood-fed females in comparison to newly-emerged ones (Martins *et al.,* 2011b). In *C. ethlius* (Lepidoptera) larvae, these plasma membrane invaginations are described as a reticular system (Locke, 1969; Jackson and Locke, 1989), however it does not seems to occur in other insects such as *G. bimaculatus* (Romer, 1974).

The peroxisome-like organelles are closely associated with SER and scattered throughtout the cytoplasm of *L. maderae* embryo (Rinterknecht, 1985) and in nymphs of *G. bimaculatus* the SER is associated to microbodies that shows no electrondense content, and reaches a diameter of up to 0.5 µm and transitions from tubules to vesicles are also found. These microbodies show peroxidase positivity demonstrated by benzidine oxidation (Romer, 1974).

Other intriguing structures are found in the oenocytes cytoplasm of insect larvae and adults. They correspond to clefts and may extend through the entire cell and extend for considerable distances and are interpreted as lipid deposits (Romer, 1974). Clark and Dahm (1973) reported that exposure of *M. domestica* adults to phenobarbital, led to the formation of membrane-like scrolls at 48 and 72 h. These membrane-like scrolls resemble to the clefts described in oenocytes of other insects (Locke, 1969; Romer, 1974) and probably are made of lipids or lipoproteins related to the SER for export, as suggested for *C. ethlius* larvae (Locke, 1969).

The isolation and degeneration of parts of the oenocyte cytoplasm takes place by the formation of autophagic vacuoles that include parts of the SER and mitochondria. The autophagosomes dynamics in the first and second instar of *G. bimaculatus* and *C. ethlius* larvae are discussed in details by Romer (1974) and Locke (1969), respectively.

Oenocytes function, biochemistry and gene expression

In spite of studies pointing to oenocytes as lipid processing cells (Gutierrez *et al.,* 2007; Martins *et al.,* 2011a), their role is not limited only to this function. Oenocytes have been shown to participate in homeostasis (*e.g.*, detoxification of xenobiotics) (Clark and Dahm, 1973; Lycett *et al.,* 2006), in the synthesis of several long chain hydrocarbon sex pheromones (Wicker-Thomas *et al.*, 2009) and other cuticle components (Fan *et al.,* 2003), and innate immunity (Martins *et al.,* 2011a). Oenocytes also play a role in the differentiation of neurons during *D. melanogaster* embryogenesis through the secretion of semaphorin (Sema2a), a peptide that drives axon elongation (Bates and Whitington, 2007).

Other studies have indicated that oenocytes produce lipids related to the impermeabilization of the insect's body (reviewed by Lockey, 1988). For instance, in *R. prolixus* nymphs, oenocytes are associated with epidermis through cell prolongations in which lipid transport from oenocytes to epidermal cells is thought to occur (Wigglesworth, 1988), and in *C. ethlius* larvae, oenocytes are located near epidermal wax glands, suggesting that they participate in the synthesis of wax precursors (Locke, 1969).

Gutierrez *et al.* (2007) suggested that the oenocytes' analogous liver function in storage of sugar and in lipid processing appear to be divided between the fat body trophocytes and oenocytes in *D. melanogaster* larvae. The lipid mobilization during starvation of fruit fly larvae led to a rise in lipid droplets within oenocytes, which maintained a low level of lipids in the hemolymph. This is similar to what happens with the adipose tissue and the liver in human during steatosis or adipose degeneration. However, unlike the mammalian liver, *D. melanogaster* oenocytes are distributed in discreet paired clusters along the larval body wall allowing the cells to be in intimate contact with the hemolymph where nutrients circulate (reviewed by Bharucha, 2009).

It has been shown that oenocytes participate directly during courtship behavior in *D. melanogaster* adults by means of the hydrocarbons they secrete, and in the female fruit flies these cells are the primary organ for communicating species and sex identity to males (Ferveur *et al.*, 1997; Billeter *et al.*, 2009). Through an elegant set of experiments, Billeter *et al.* (2009) generated an oenocyte Gal4 driver derived from the regulatory sequence of one of the desat1 promoters expressed specifically in oenocytes of adult females. Ablation of adult oenocytes in males by inducing expression of the pro-apoptotic gene *hind* suggested that the oenocyte-less (oe^-) males display normal courtship behavior towards wild-type females, but in a fashion considered less intense than control males. However, wild-type females are less receptive to oe^- males than control males (Billeter *et al.*, 2009). The oenocytes ablation induced an unnatural behavior in males that have vigorous courtship by each other (Ferveur *et al.*, 1997). Surprisingly, oe^- females, *i.e.*, lacking hydrocarbons, are more attractive than those

with a normal hydrocarbon profile to wild males, suggesting that female hydrocarbons normally act to slow down male mating attempts. Billeter *et al.* (2009) also tested the behavior of males from other species towards *D. melanogaster* or females. They showed that males of other *Drosophila* species (such as *D. simulans*, *D. yakuba* and *D. erecta*) engaged in courtship of the *D. melanogaster* oe$^-$ females, but exhibit limited or no courtship towards control. These data indicated that oenocytes and their hydrocarbon products are important components of the reproductive isolation barrier.

The synthesis of pheromone in the adult of *D. melanogaster* has been demonstrated to be a result of desaturase activity expressed mainly in the oenocytes. Wicker-Thomas *et al.* (2009) used RNAi to knock down a desaturase gene (*desat1*) in oenocytes resulting in 96 % decrease in unsaturated hydrocarbons in adult males and 78 % in females. Inactivation of the female specific *desatF* gene (responsible for diene formation), resulted in a dramatic loss of pheromones (98 %) combined witha two-fold increase in monoenes.

Another gene whose expression is prominent in the fat body, in oenocytes, and in midgut tissues of *D. melanogaster* is *Indy* (for "*I'm not dead yet*") (Knauf *et al.,* 2002). Oenocytes are supposedly related to fruit fly longevity and, interestingly, a reduction in INDY proteins levels dramatically extended the life span in fruit flies without sacrificing their fertility or physical activity (Knauf *et al.,* 2002). Also, the expression of catalase in *D. melanogaster*is mostly confined to these same tissues indicated above, starting from the embryonic development and extending into adult stages, and the expression levels rise according to age (Klichko *et al.,* 2004).

Oenocytes seem to be an important site for the biosynthesis of hemoproteins in insects. In the *D. melanogaster* embryo, a site for porphyrin biosynthesis, and expression of the enzyme δ-aminolevulinate synthase (ALAS) is specifically detected in oenocytes suggesting a role of these cells in hemoproteins biosynthesis (Ruiz de Mena *et al.,* 1999). Our group previously investigated the transcriptome of oenocytes isolated from *A. aegypti* pupae (Martins *et al.,* 2011a). Our data indicated that eight percent of the transcripts in these oenocytes coded for the hemoprotein cytochrome P450. This enzyme participates in the metabolism of different molecules such as sterols, steroid hormones, and several lipids, and also in a variety of physiological roles ranging from development, to feeding and growth, to resistance to pesticides and tolerance to plant toxins [Martins *et al.* (2011a) and papers therein]. Accordingly, P450 expressed in oenocytes likely participate in steroid metabolism as previously suggested by Romer *et al.* (1974) demonstrating that oenocytes isolated from *T. molitor* larvae synthesize α- and β-ecdysone from 4-^{14}C-cholesterol precursors *in vitro*.

In addition, approximately four percent of the transcripts in the oenocytes from *A. aegypti* pupae encoded other types of detoxification proteins, such as alcohol dehydrogenase, catalase, and a NADPH cytochrome P450 reductase (CPR) (Martins *et al.*, 2011a). In *Anopheles gambiae*, oenocytes are one of the major sites of xenobiotic metabolism in the adult mosquito (Lycett *et al.,* 2006). In both *A. gambiae* and *D. melanogaster*, oenocytes express high levels of NADPH cytochrome P450 reductase (CPR) that is required for cytochrome P450s involved in metabolic insecticide resistance, and RNAi mediated knockdown of CPR in oenocytes led to enhanced sensitivity to permethrin (Lycett *et al.,* 2006). *Aedes aegypti* oenocytes also express 23 transcripts that code for lipid-metabolizing proteins, such as fatty acid synthase (FAS), elongase and estradiol dehydrogenase. These enzymes are related to integument hydrocarbon synthesis, such as long-chain fatty acids, and pheromone synthesis [Martins *et al.* (2011a) and papers therein]. Such findings are in agreement with results obtained by Fan *et al.* (2003) showing that oenocytes are responsible for hydrocarbon synthesis in *B. germanica.* Interestingly, a small fraction of *A. aegypti* transcripts (1.9 %) coding for proteins such as lysozymes and serine proteases that likely participate in mechanisms of innate immune responses, is suggestive of a role for oenocytes in insect immunity.

Hydrocarbon synthesis by oenocytes depends on age (Hepburn *et al.,* 1991). In this case, honeybee (*A. mellifera*) workers displayed an increase in saturated C25 and C27 hydrocarbons with a concomitant decrease in the C33 molecules present in oenocytes as they become older. In addition, the hydrocarbon and fatty acid profiles of isolated oenocytes were also found in newly synthesized wax, suggesting that oenocytes are the likely source of hydrocarbons for this cuticular component (Hepburn *et al.,* 1991).

Additional studies using labeled molecules to investigate the metabolism in oenocytes were performed by Diehl (1973, 1975), Tobe and Davey (1974), and Romer (1980). For example, using H^3-tyrosine and -leucine injected in *G. austeni* females Tobe and Davey (1974) demonstrated that the incorporation of these molecules by oenocytes is age dependent, and is highest at eclosion and just after larviposition. In other insect models, such as in fifth-instar nymphs of *S. gregaria*, oenocytes synthesize and secret hydrocarbons, mainly cuticular lipids, from ^{14}C-acetate and several other lipids of unknown nature (Diehl, 1973, 1975). Also, incorporation of ^{14}C-labeled cholesterol following abdominal injections of *M. domestica* larvae indicated a strong labeling of certain portions of the fat body especially within oenocytes suggesting that these cells play a central role in the metabolism and/or storage of sterols and they possibly take part in abdominal ecdysone biosynthesis (Studinger and Willig, 1975).

Oenocytes infection

Little is known about the ability of pathogens to infect insect oenocytes. Generally speaking, question such as the type of pathogens that are able to invade these cells, or how invasion and establishment of infection proceeds (in case of those pathogens shown able to infect), as well as changes or responses to infection by oenocytes, have never been fully addressed. Nevertheless, it is

known that Microsporidia fungi are able to infect mosquito oenocytes, including larvae, pupae and imago, and that these infections occur transovarially (Kellen *et al.,* 1965, 1967; Hall, 1985; Sweeney *et al.,* 1988).

Among the different fungi species that were shown to infect oenocytes (of mosquitoes) we include those in the genera *Thelohania*, *Amblyospora, Nosema*, *Stempellia, Parathelohania* and *Plistophora* (Kellen *et al.,* 1967; Hall, 1985; Sweeney *et al.,* 1988; Garcia *et al.,* 1993). Infection of oenocytes can sometimes be recognized, depending of the mosquito species or the invading fungi, as the oenocytes hypertrophied as the parasites multiply, and infections are fatal to the insect (Kellen *et al.,* 1965). However, in females of *Culex salinarius* (Diptera) infected by *Amblyospora* sp., parasites do not multiply significantly until late pupal or early adult stages at which time they gradually fill the oenocytes (Hall, 1985). Hall (1985) also reported that in healthy mosquitoes, the larval oenocytes break down during the pupal and early adult stages, but in infected individuals they persist, undergoing fusion during the pupal stage to form syncytia containing two to seven nuclei per syncytial oenocyte. During this period, the infected oenocytes break loose from the fat body and begin circulate throughout the hemocel, mainly in the head and particularly the thorax in the vicinity of the foregut (Hall, 1985).

Infected hypertrophied oenocytes of *Anopheles pseudopunctipennis franciscanus* adults adhered closely to the gut and harbored various stages in the life cycle of *Nosema chapmani.* In larvae and adults of *Culex tarsalis* infected by *Nosema lunatum*, oenocytes were especially evident in adult females, where they occasionally attached to ovaries and fat body (Kellen *et al.,* 1967).

Amblyospora sp. in *C. salinarius* exhibited two distinct developmental stages, one in each host sex. In females, the entire life cycle was restricted to host oenocytes where multiplication of diplokarya occurred during merogony (Andreadis, 1978). Infections were initiated when small binucleated sporoplasms infected the developing eggs within the female host and were subsequently transferred to the next generation when the eggs were laid. Within embryonated eggs and newly hatched larvae of both sexes small, oval, diplokaryotic stages invaded host oenocytes and underwent an initial multiplicative phase (merogony) where they divided mitotically to produce more diplokarya. Diplokarya subsequently broke out of the oenocytes and invaded trophocytes where they multiplied repeatedly (Andreadis,1978).

In *Anopheles quadrimaculatus,* diplokaryotic meronts of *Parathelohania anophelis* (Microspora: Amblyosporidae) were observed within oenocytes of third-instar female larvae. Parasites containing six to 40 nuclei were also noted in oenocytes from the second and third abdominal segments of female mosquitoes 24 h after emergence. From larvae to newly emerged adults, parasites multiplied within the oenocytes restricted to the second abdominal segment and in the anterior of the third abdominal segment. The development of the spore took place inside large groups of oenocytes in the interior of the abdomen during days three to five after emergence and before the female took a blood meal (Garcia *et al.,* 1993).

The infection of oenocytes by viruses has been poorly studied, and little data are available. Nevertheless, it has been reported that old adults of *D. melanogaster* oenocytes were supposedly infected by unknown virus-like particles (Philpott *et al.,* 1969), whereas Dengue virus serotype 2 was able to infected oenocytes isolated from *A. aegypti* in culture (Guedes and Pimenta, 2009).

Oenocytes isolation and culture

Until recently, very few studies involving isolated insect oenocytes were reported. Today, with the improvement of dissection and harvesting techniques *in vitro*, oenocyte cultures can be established opening new avenues of research focused on the role of these cells in insect metabolism.

The ability to isolate and maintain oenocytes *in vitro* led to the first studies demonstrating the role of these cells in the metabolism of ecdsteroids. This was accomplished by Romer *et al.* (1974) using oenocytes isolated from the abdomen of *T. molitor* larvae that demonstrated that these cells are able to synthesize α- and β-ecdysone from ^{14}C-labeled cholesterol. Oenocytes were also successfully isolated from *B. germanica* and found to represent different cell types (Fan *et al.*, 2003). Using a mild enzymatic treatment these authors obtained oenocytes in suspension after digesting their basal lamina. From this highly oenocyte-enriched cell suspension they demonstrated that oenocytes can produce hydrocarbons.

Although obtaining isolated oenocytes can be tricky due to the nature of how these cells are distributed in the insect and how they might associate with other tissues, oenocytes in the mosquito pupa are easily separated and collected (Martins *et al.*, 2011a*,* d). Additionally, a detailed demonstration on how to identify and collect oenocyte clusters from *D. melanogaster* adults can be found at Krupp and Levine (2010).

Studies involving the primary cultivation of insect oenocytes are rare and one example is the work of Martins *et al.* (2011d). Maintaining viable in culture for up to two months they described details about the morphology of these cells combining various microscopic approaches. These cells were attached to the glass substrate and were visualized as single or clustered cells that maintain main cytoplasmic characteristics found in freshly isolated cells, such as the general chromatin organization and the cytoplasm filled with SER. However, there is a decrease in the mitochondria number and size in the cultured cells. During this cultivation period some cells keep their basal lamina and do not divided and this result was expected since oenocytes are highly differentiated and specialized cells. The absence of proliferation occurs also in oenocytes from *D. melanogaster* embryo's *in vitro*. However, in this case, oenocytes are capable to aggregate after dissociation (Lesseps, 1965), what not happens with pupal oenocytes of *A. aegypti* (Martins *et al.,* 2011d).

Clearly, methods to isolate and maintain oenocytes *in vitro* can contribute towards studies aimed at understanding the metabolism of such cell type, perhaps providing novel strategies for insect control. Further, the long-term survival of viable oenocytes in primary culture also provides a tool for investigating their interactions with pathogens (Martins *et al.,* 2011d).

Conclusions and perspectives

This review was intended to provide the reader with a general perspective of oenocyte function by outlining some of the key findings related to oenocyte's location within insects, histochemistry, biochemistry, and gene expression. With the use of advanced molecular tools it has become clear that insect oenocytes play a wider role during insect metabolism than lipid processing. The identification of proteins (or of transcripts) expressed within oenocytes demonstrated that these cells also participate in detoxification. In addition, little is known about the role(s) of different oenocyte populations during insect post-embryonic development, including larval and imaginal stages. Gene ablation targeting *D. melanogater* oenocytes in larval stages indicate that these cells can be considered analogous to mammalian hepatocytes (see Gutierrez *et al.,* 2007). On the other hand, in adult fruit flies, oenocytes play key role in the sexual behavior by means the synthesis of hydrocarbons (Ferveur*et al.*, 1997; Billeter *et al.*, 2009). However, such oenocyte functions in *D. melanogaster* have not yet been confirmed for other insects.

Thus, in our view, the ability to harvest and maintain oenocytes in culture can positively impact future studies focused on oenocytes metabolism, and gene and protein expressions, and assist in identifying other potential roles in these cells so crucial for insect survival.

References

Agui N. Joint action of prothoracic glands and oenocytes on the cultivated wing discs of the cabbage armyworm, *Mamestra brassicae* L. *in vitro* (Lepidoptera: Noctuidae). Appl. Ent. Zool. 9: 256-260, 1974.

AndreadisTG. Life cycle and epidemiology of *Aniblyospora* sp. (Microspora: Thelohaniidae) in the mosquito *Culex salinarius* Coquillett. Dissertation, University of Florida, 1978.

Bates KE, Whitington PM. Semaphorin 2a secreted by oenocytes signals through plexin B and plexin A to guide sensory axons in the *Drosophila* embryo. Dev. Biol. 302: 522-535, 2007.

Bharucha KN. The epicurean fly: using *Drosophila melanogaster* to study metabolism. Pediatr. Res. 65: 132-137, 2009.

Billeter JC, Atallah J, Krupp JJ, Millar JG, Levine JD. Specialized cells tag sexual and species identity in *Drosophila melanogaster*. Nature 461: 987-992, 2009.

Burns KA, Gutzwiller LM, Tomoyasu Y, Gebelein B. Oenocyte development in the red flour beetle *Tribolium castaneum*. Dev. Genes Evol. 222: 77-88, 2012.

Camargo-Mathias MI, Fontanetti CS. Ultrastructural features of the fat body and oenocytes of *Rhinocricus padbergi* Verhoeff (Diplopoda, Spirobolida). Biocell 24: 1-12, 2000.

Clark MK, Dahm PA. Phenobarbital-induced, membrane-like scrolls in the oenocytes of *Musca domestica* Linnaeus. J. Cell. Biol. 56: 870-875, 1973.

Coupland RE. Observations on the normal histology and histochemistry of the fat body of the locust (*Schistocerca gregaria*). J. Exp. Biol. 34: 290-296, 1975.

Diehl PA. Paraffin synthesis in the oenocytes of the desert locust. Nature 243: 468-470, 1973.

Diehl PA. Synthesis and release of hydrocarbons by the oenocytes of the desert locust *Schistocerca gregaria*. J. Insect Physiol. 21: 1237-1246, 1975.

Dorn A, Romer F. Structure and function of prothoracic glands and oencoytes in embryos and last larval instars of *Oncopeltus fasciatus* Dallas (Insecta, Heteroptera). Cell Tissue Res. 171: 331-350, 1976.

Dunkel FV, Mallory BM. Studies on the internal anatomy of the black carpet beetle, *Attagenus megatoma*. Ann. Entomol. Soc. Am. 61: 755-765, 1968.

Elstob PR, Brodu V, Gould AP. Spalt-dependent switching between two cell fates that are induced by the *Drosophila* EGF receptor. Development 128: 723-732, 2001.

Evans JJT. Development and ultrastructure of the fat body cells and oenocytes of the queensland fruit fly, *Dacus tryoni* (Frogg.). Z. Zellforsch. Mikrosk. Anat. 81: 49-61, 1967.

Fan Y, Zurek L, Dykstra MJ, Schal C. Hydrocarbon synthesis by enzymatically dissociated oenocytes of the abdominal integument of the german cockroach *Blattella germanica*. Naturwissenschaften 90: 121-126, 2003.

Ferveur JF, Savarit F, O'Kane CJ, Sureau G, Greenspan RJ, Jallon JM. Genetic feminization of pheromones and its behavioral consequences in *Drosophila* males. Science 276: 1555-1558, 1997.

Fontanetti CS, Camargo-Mathias MI, Tiritan BMS. The fat body in *Rhinocricus padbergi* (Diplopoda, Spirobolida). Iheringia Sér. Zool. 94: 351-355, 2004.

Garcia JJ, Hazard EI, Fukuda T. Light and electron microscopy studies on the development of *Parathelohania anophelis* (Microspora: Amblyosporidae) in female *Anopheles quadrimaculatus* (Diptera: Culicidae). J. Invertebr. Pathol. 61: 86-89, 1993.

Gould AP, Elstob PR, Brodu V. Insect oenocytes: a model system for studying cell-fate specification by Hox genes. J. Anat. 199: 25-33, 2001.

Guedes BAM, Pimenta PFP. First ultrastructural demonstration of dengue virus interaction with *Aedes aegypti*oenocytes. XXII Congresso Da SBMM. 2009. http://actamicroscopica.ivic.gob.ve/uploads/Suplementos/Vol_18_Supp_B_2009_Memorias_SBMM_2009/files/posteres3b57.html?track=B12.

Gutierrez E, Wiggins D, Fielding B, Gould AP. Specialized hepatocyte-like cells regulate *Drosophila* lipid metabolism. Nature 445: 275-280, 2007.

Hall DW. The distribution of *Amblyospora* (Microspora) sp.-infected oenocytes in adult female *Culex salivarius*: significance for mechanism of transovarial transmission. J. Am. Mosq. Control. Assoc. 1: 514-515, 1985.

Hartenstein AY, Rugendorff A, U Tepass, Hartenstein V. The function of the neurogenic genes during epithelial development in the *Drosophila* embryo. Development 116: 1203-1220, 1992.

Hepburn HR, Bernard RTF, Davidson BC, Muller WJ, Lloyd P, Kurstjens SP, *et al.* Synthesis and secretion of beeswax in honeybees. Apidologie 22: 21-36, 1991.

Imms AD. On the larval and pupal stages of *Anopheles maculipennis*, Meigen. J. Hyg. 7: 291-318, 1907.

Jackson A, Locke M. The formation of plasma membrane reticular systems in the oenocytes of an insect. Tissue Cell 21: 463-473, 1989.

Jensen PV, Børgesen LW. Regional and functional differentiation in the fat body of pharaoh's ant queens, *Monomorium pharaonis* (L.). Arthrop. Struct. Dev. 29: 171-184, 2000.

Johson MB, Butterworth FM. Maturation and aging of adult fat body and oenocytes in *Drosophila* as revealed by light microscopic morphometry. J. Morphol. 184: 51-59, 1985.

Juárez MP, Fernández GC. Cuticular hydrocarbons of triatomines. Comp. Biochem. Physiol. 147A: 711-730, 2007.

Kellen WR, Clark TB, Lindegren JE. Two previously undescribed *Nosema* from mosquitoes of California (Nosematidae: Microscoporidia). J. Invertebr. Pathol. 9: 19-25 1967.

Kellen WR, Harold CC, Clark TB, Lindegren JE. Host-parasite relationships of some *Thelohania* from mosquitoes (Nosematidae: Microsporidia). J. Invertebr. Pathol. 7: 161-166, 1965.

Klichko VI, Radyuk SN, Orr WC. Profiling catalase gene expression in *Drosophila melanogaster* during development and aging. Arch. Insect Biochem. Physiol. 56: 34-50, 2004.

Knauf F, Rogina B, Jiang Z, Aronson PS, Helfand SL. Functional characterization and immunolocalization of the transporter encoded by the life-extending gene Indy. Proc. Natl. Acad. Sci. USA. 99: 14315-14319, 2002.

Koschevnikov GA. Ueber den fettkörper und die oenocyten der honigbiene. Zool. Anz. 13: 337, 1900.

Krupp JJ, Levine JD. Dissection of oenocytes from adult *Drosophila melanogaster*. J. Vis. Expe. 18(41): pii: 2242. doi: 10.3791/2242. 2010.

Kuterbach DA, Reeder RJ, Frankel RB. Iron-containing cells in the honey bee (*Apis mellifera*). Science 218: 695-697, 1982.

Landois L. Ueber die funktion des fettkörpers. Zeitschr. f. Wissensch. Zoologie 15: 371-372, 1865.

Lawrence PA, Johnston P. Cell lineage of the *Drosophila* abdomen: the epidermis, oenocytes and ventral muscles. J. Embryol. Exp. Morph. 72: 197-208, 1982.

Lesseps RJ. Culture of dissociated *Drosophila* embryos: aggregated cells differentiate and sort out. Science 148: 502-503, 1965.

Locke M. The ultrastructure of the oenocytes in the molt/intermolt cycle of an insect. Tissue Cell 1: 103-154, 1969.

Lockey KH. Lipids of the insect cuticle: origin, composition and function. Comp. Biochem. Physiol. 89B: 595-645, 1988.

Lycett GJ, McLaughlin LA, Ranson H, Hemingway J, Kafatos FC, Loukeris TG, *et al. Anopheles gambiae* P450 reductase is highly expressed in oenocytes and *in vivo* knockdown increases permethrin susceptibility. Insect Mol. Biol. 15: 321-327, 2006.

Martins GF, Ramalho-Ortigão JM, Lobo N, Severson DW, McDowel MA, Pimenta PFP. Insights into the transcriptome of oenocytes from *Aedes aegypti* pupae. Mem. Inst. Oswaldo Cruz 106: 308-315, 2011a.

Martins GF, Serrão JE, Ramalho-Ortigão JM, Pimenta PFP. Hystochemical and ultrastructural studies of the mosquito *Aedes aegypti* fat body: effects of aging and diet type. Micro. Res. Tech. 4: 1032-1039, 2011b.

Martins GF, Serrão JE, Ramalho-Ortigão JM, Pimenta PFP. A comparative study of fat body morphology in five mosquito species. Mem. Inst. Oswaldo Cruz 106: 742-747, 2011c.

Martins GF, Guedes BAM, Silva LM, Serrão JE, Fortes-DiasCL, Ramalho-Ortigão JM, *et al.* Isolation, primary culture and morphological characterization of oenocytes from *Aedes aegypti* pupae. Tissue Cell 43: 83-90, 2011d.

Paes-de-Oliveira VT, Cruz-Landim C. Histological and ultrastructural aspects of the fat body in virgin and physogastric queens of *Melipona quadrifasciata anthidioides* Lepeletier, 1836 (Hymenoptera, Apidea, Meliponini). Braz. J. Morphol. Sci. 23: 385-392, 2006.

Philpott DE, Weibel J, Atlan H, Miquel J. Viruslike particles in the fat body, oenocytes, and central nervous tissue of *Drosophila melanogaster* imagoes. J. Invertebr. Pathol. 14: 31-38, 1969.

Rinterknecht E, Matz G. Oenocyte differentiation correlated with the formation of ectodermal coating in the embryo of a cockroach. Tissue Cell 15: 375-390, 1983.

Rinterknecht E. Cuticulogenesis correlated with ultrastructural changes in oenocytes and epidermal cells in the late cockroach embryo. Tissue Cell 17: 723-743, 1985.

Roma GC, Camargo-Mathias MI, Bueno OC. Fat body in some genera of leaf-cutting ants (Hymenoptera: Formicidae). Proteins lipids and polysaccharides detection. Micron 37: 234-242, 2006.

Roma GC, Bueno OC, Camargo-Mathias MI. Chemical detection of the proteins and lipids in the fat body cells from workers of Attini ants (Hymenoptera: Formicidae). Cell. Biol. Int. 32: 406-416, 2008.

Roma GC, Bueno OC, Camargo-Mathias MI. Morpho-physiological analysis of the insect fat body: a review. Micron 41: 395-401, 2010.

Romer F. Ultrastructural changes of the oenocytes of *Gryllus bimaculatus* DEG (Saltatoria, Insecta) during moulting cycle. Cell Tissue Res. 151: 27-46, 1974.

Romer F. Histochemical and biochemical investigations concerning the function of larval oenocytes of *Tenebrio molitor* L. (Coleoptera, Insecta). Histochemistry 69: 69-84, 1980.

Romer F, Gnatzy W. Arachnid oenocytes: ecdysone synthesis in the legs of harvestmen (Opilionidae). Cell Tissue Res. 216: 449-453, 1981.

Romer F, Emmerich H, Nowock J. Biosynthesis of ecdysones in isolated prothoracic glands and oenocytes of *Tenebrio molitor in vitro*. J. Insect Physiol. 20:1975-1987, 1974.

Roth LM. The oenocytes of *Tenebrio*. Ann. Entomol. Soc. Am. 35: 81-84, 1942.

Ruiz de Mena I, Fernandez-Moreno MA, Bornstein B, Kaguni LS, Garesse R. Structure and regulated expression of the delta-minolevulinate synthase gene from *Drosophila melanogaster*. J. Biol. Chem. 274: 37321-37328, 1999.

Ruvolo MCC, Cruz-Landim C. Morphologic and morphometric aspects of oenocytes of *Apis mellifera* queens and workers in different phases of life. Mem. Inst. Oswaldo Cruz 88: 387-395, 1993.

Ruvolo MCC, Cruz-Landim C. Quantitative analysis of the relative volume occupied by oenocytes in the parietal fat body and wax epithelium development in *Apis mellifera* workers. Revta. Bras. Ent. 39: 111-114, 1995.

Saxena AK, Agarwal GP. Oenocytes of poultry lice *Lipeurus lawrensis tropicalis* Peters (Phthiraptera: Ischnocera). Experientia 36: 68, 1980.

Snodgrass RE. Principles of insect morphology. McGraw-Hill Book Co., Inc. New York and London, 1935.

Sohal SR. Fine Structural alterations with age in the fat body of the adult male housefly, *Musca domestica*. Z. Zellforsch. 140: 169-175, 1973.

Souza TS, Angelis DF, Fontanetti CS. Histological and histochemical analysis of the fat body of *Rhinocricus padbergi* (Diplopoda) exposed to contaminated industrial soil. Water Air Soil Pollut. 221: 235-244, 2011.

Stoppie P, Briers T, Huybrechts R, De Loof A. Moulting hormone, juvenile hormone and the ultrastructure of the fat body of adult *Sarcophaga bullata* (Diptera). Cell Tissue Res. 221: 233-244, 1981.

Studinger G, Willig A. Biosynthesis of α- and β-ecdysone in isolated abdomens of larvae of *Musca domestica*. J. Insect Physiol. 21: 1793-1798, 1975.

Sweeney AW, Graham MF, Hazard EI. Life cycle of *Amblyospora dyxenoides* sp. nov. in the mosquito *Culex annulirostris* and the copepod *Mesocyclops albicans*. J. Invertebr. Pathol. 51: 46-57, 1988.

Symonová R, Smrž J. First record of hemocytes and oenocytes in freshwater ostracodes. J. Crust. Biol. 29: 18-25, 2009.

Tadkowski TM, Jones JC, Firman J. The fine structure of the imaginal oenocytes of *Aedes aegypti.* Ann. Entomol. Soc. Am. 70: 837-840, 1977.

Thiele T, Camargo-Mathias MI. Morphology, ultramorphology and morphometry of the fat body of virgin females and queens of the ants *Pachycondyla striata* (Hymenoptera, Formicidae). Sociobiology 42: 234-254, 2003.

Tobe SS, Davey KG, Huebner E. Nutrient transfer during the reproductive cycle in *Glossina austeni* Newst. I. Histology and histochemistry of the milk gland fat body and oenocytes. Tissue Cell 5: 633-650, 1973.

Tobe SS, Davey GK. Autoradiographic study of protein synthesis in abdominal tissues of *Glossina austeni*. Tissue Cell 6: 255-268, 1974.

Vashchenok VS. Morphological and physiological changes in *Echidnophaga oshanini* Wagn. (Aphaniptera, Pulicidae) during feeding and reproduction. Entomol. Obozr. 45: 4040-410, 1966.

Vickery RK. Evidence of a protoplasmic network in the oenocytes of the silkworm. Ann. Entomol. Soc. Am. 8: 285-290, 1915.

Wicker-Thomas C, Guenachi I, Keita YF. Contribution of oenocytes and pheromones to courtship behaviour in *Drosophila*. BMC Biochemistry 10: 21, 2009.

Wigglesworth VB. The physiology of the cuticle and of ecdysis in *Rhodnius prolixus* (Triatomidae, Hemiptera) with special reference to the function of the oenocytes and of the dermal glands. Q. J. Microsc. Sci. 76: 269-319, 1933.

Wigglesworth VB. The storage of protein fat glycogen and uric acid in the body and other tissues of mosquito larvae. J. Exp. Biol. 19: 56-77, 1942.

Wigglesworth VB. The source of lipids and polyphenols for the insect cuticle: the role of fat body, oenocytes and oenocytoids. Tissue Cell 20: 919-932, 1988.

Zara FJ, Caetano FH. Ultramorphology and histochemistry of fat body cells from last instar larval of the *Pachycondyla (=Neoponera) villosa* (Fabricius) (Formicidae: Ponerinae). Braz. J. Biol. 64:725-735, 2004.