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Investigation of Hemocyte Types in *Lepisma saccharina*

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Abstract
Hemocytes are specialized hemolymph cells found in insects, responsible for a variety of important physiological functions such as immunity, wound healing, and the regulation of the insect's circulatory system. The study of insect hemocytes has become increasingly important in recent years due to the emergence of insect-borne diseases and the need to develop new strategies for insect control. Understanding the biology of insect hemocytes has the potential to lead to the development of novel insecticides and strategies for controlling insect-borne diseases. The aim of this study was to examine the hemocytes in *Lepisma saccharina* to determine if there were any variations in the types of hemocytes present among ametabolous and holometabolous insect or in comparison to other arthropods. Hemolymph of *L. saccharina* collected and hemolymph smears were stained with Wright's stain. In adult *L. saccharina* two hemocyte types, prohemocyte and plasmatocyte were observed. The hemocyte types were identified based on their size, presence or absence of granules, and the ratio of their nucleus to cytoplasm, using light microscopy.

Keywords: Hemolymph, insect, prohemocyte, plasmatocyte, granulocyte, oenocytoid

1. Introduction

Most species on Earth are insects, and thus understanding their evolutionary relationships is key to understanding the evolution of life. (Trautwein et al., 2012). *Lepisma saccharina*, commonly known as the silverfish, is a small, wingless insect found throughout the world. It is a primitive insect that has remained largely unchanged for millions of years. With its distinctive silvery scales and elongated body (van Gelderen et al., 2010; Joshi et al., 2020), it is an intriguing creature that has fascinated scientists and casual observers alike for centuries. *L. saccharina* has even been incorporated into medical treatments (Zimian et al., 1997). The features of the immune system of this ancient and interesting speciesare also quite remarkable.

Immunity is a response to environmental conditions. From the moment life began on Earth, immune mechanisms have also begun to develop. Insects, as invertebrates, lack certain important immune mechanisms found in vertebrates. The most significant of these is the production of antibodies specifically developed for antigens, a form of defense known as acquired immunity. While insects do not have such a robust immune system, they are not completely defenseless in the external world (Zhang et al., 2015). In 1918, Rudolf W. Glaser revealed for the first time that grasshoppers had immune systems (Glaser, 1918).

Unlike vertebrates, insects do not exhibit antigen-specific immune responses. These immune responses are mediated by antibodies produced in vertebrate animals against microorganisms. This defense system, known as adaptive or acquired immunity, is absent in insects, as in all invertebrates. However, the absence of antibody production does not mean that invertebrates lack defenses against microorganisms. In fact, they can defend themselves, primarily through their innate immune system. Innate immunity can occur at both the cellular and humoral levels, utilizing body fluids circulating between cells. Various mechanisms exist to fend off microbial spread (Zhang et al., 2015).

When vertebrates and invertebrates are compared in terms of immune system, body fluids in vertebrates are mostly found in a closed circulation within the blood and lymphatic vessels, while invertebrates have an open circulation. In invertebrates with an open circulatory system, such as insects, body fluids are called hemolymph because there is no separation of blood and lymphatic fluids as seen in vertebrates. Therefore, there are different mechanisms in invertebrates to prevent the spread of microbes. Hemocytes mediate defense mechanisms such as phagocytosis, capsule formation and coagulation (Lackie, 1988; Strand and Pech, 1995; Gillespie et al., 1997; Irwing et al., 2005). Encapsulation is an immune response wherein insects protect themselves from multicellular parasites such as nematodes. During encapsulation, specific insect hemocytes are attracted to foreign invaders and accumulate on their surfaces (Levin, 2007). This process effectively traps the invader in a capsule of hemocytes, rendering the parasite harmless to the insect host.

In humoral immunity, when compared especially with vertebrates, the lipid and glycogen-rich fat body, which is a structure reminiscent of the liver in terms of its function, takes part in insects (Larsen, 1976). Here, different mechanisms such as coagulation, formation of melanin pigment and production of peptides with antimicrobial properties can be mentioned.

The main factor in the evolutionary success of insects is their effective immune system that allows them to fight pathogens (Morley et al., 2013). Insects do not have an adaptive immune system capable of specifically recognizing and responding to particular pathogens. Instead, they possess various physical, chemical, and cellular mechanisms that work together to protect them from infection (Owens, 2019). One important adaptive ability of insects that facilitates their success is the plasticity of their immune system. Although they only

have innate immune mechanisms, insects can increase their resistance after the first encounter with the pathogen (Sułek et al., 2021). Insects possess a variety of hemocyte types that play important roles in their immune system. They are responsible for recognizing, engulfing, and killing pathogens. The most common types of hemocytes in insects are plasmatocytes, granulocytes, oenocytoids, spherulocytes, adipohemocytes and coagulocytes (Kwon et al., 2014).

In this context, research on insect immunity has become increasingly significant, with scientists making great strides in unraveling the mysteries of this ancient and complex system. This study was conducted to analyze *L. saccharina*, an ametabolous insect species, in terms of hemocyte types.

2. Material and Methods

In this study, samples of *Lepisma saccharina* from the Lepismatidae family were examined. *Lepisma saccharina* samples were collected as a result of field surveys in and around Iznik during March and April. A total of 15 samples were used in the study. Hemolymph needed for the measurement and examination of hemocytes was obtained by cutting the femur at the joint connecting it to the thorax using small scissors. The hemolymph was collected into heparinized hematocrit capillary tubes. Hemocyte measurements and counts were performed using smears stained with Wright's dye.

Preparation of the smear: A drop of hemolymph fluid was dripped 1 cm from one edge of a pre-cleaned slide. A second clean slide was taken as a spreader and adjusted to make a 25^{o} angle with the hemolymph drop, the spreading was performed by pushing the spreading slide at the same speed and left to dry. Staining was performed according to the staining cup method. Accordingly, well spread preparates were selected and placed on the shelf of the staining cup. It was waited for one minute by dropping enough (10-12 drops) of Wright's dye was added enough (10-12 drops) to be spread on the slide and 10-12 drops of buffer solution were dropped and waited for 10 minutes. Then, the dye-buffer mixture was poured, shaken in distilled water and left to dry. Afterwards, the concealer was dripped and the coverslip was closed to make a permanent preparate.

Hemocyte Counting: Hemocyte type counts were performed using a Neubauer hemocytometer. Counting was conducted for each type of hemocyte detected in each sample in 25 squares with an area of 1 mm2 in the middle and the average was taken. Standard Hayem's solution was used as the dilution solution for counting hemocyte types.

3. Findings and Discussion

The first studies on hemocytes, known as insect blood cells, were conducted in 1918 by Rudolf W. Glaser, who discovered that grasshoppers had an immune system. Since then, studies on the insect immune system have primarily focused on determining hemocyte types. Initially, many different types of hemocytes were identified. Up to 30 different hemocyte types have been identified in some insects (Demirsoy, 2006). Gupta (1979) compiled a table of hemocyte types identified across various insect orders (Table 1).

Table 1. In the study conducted by Gupta (1979), hemocyte types detected in various insect orders

Takım	PR	PL	GR	SP	CO	AD	OE
Collembola	-	-	+	-	-	-	-
Thysanura	-	+	+	+	-	+	-
Epheroptera	+	+	+	-	-	-	+
Odonata	-	+	+	-	-	-	-
Orthoptera	+	+	+	+	-	-	-
Dermaptera	+	+	+	+	-	+	-
Blattaria	+	+	+	+	+	+	+
Mantodea	+	+	+	+	-	-	-
Plecoptera	+	+	+	+	-	+	-
Hemiptera	+	+	+	-	+	-	+
Hymenoptera	+	+	+	-	+	-	+
Coleoptera	+	+	+	+	+	+	+
Megaloptera	+	+	+	-	-	+	+
Neuroptera	+	+	+	+	+	-	+
Trichoptera	+	+	+	-	-	-	-
Lepidoptera	+	+	+	+	+	+	+
Diptera	+	+	+	+	+	+	+

PR=Prohemosit, PL=Plazmatosit, GR=Granülosit, CO=Koagülosit, AD=Adipohemosit, OE=Önositoit

Different numbers of hemocyte types were determined in studies on insects. Seven types of hemocyte were identified as ultra-structurally: Prohemocyte, plasmatocyte, granulocyte, spherulocyte, adipohemocyte, oenocytoid, and coagulocyte. Two more varieties were observed: vermicide and podosite. When podocytes and vermicides were first observed, they were not considered as separate hemocyte types because they were ultrastructurally similar to plasmatocytes (Devauchelle, 1971). In lepidopteran larvae, four different hemocyte types are known: plasmatocytes, which play a role in capsule formation; granulocytes, which perform phagocytosis eucytoids, which produce enzymes like phenoloxidase involved in melanin synthesis; and spherulocytes whose immune-related functions are not fully understood (Lavine and Strand, 2002). Jalali and Salehi (2008) determined a total of six hemocyte types in *Papilio demoleus* type: prohemocyte, plasmatocyte, granulocyte, spherulocyte, eocytoid, and adipohemocyte. Yelkovan et al. (2021) determined five hemocyte types in *Apis mellifera anatolica* as prohemocyte, plasmatocyte, granulocyte, adipohemocyte and oenocytoid in all castes. Öztürk et al. (2024) identified four different hemocyte types (prohemocyte, plasmatocyte, granulocyte, and oenocytoid) in *Bombyx mori* throughout its life stages.

In this study, light microscopy examinations of the hemolymph of the ametabolous species *Lepisma saccharina* revealed the presence of two main hemocyte types: prohemocytes and plasmatocytes (Figure 1). An average of 30 prohemocytes and 70 plasmatocytes were counted per 1 mm3 of hemolymph. Although the prohemocytes detected in the hemolymph are smaller in size, they have a nucleus that fills almost the entire cell. The average diameter of prohemocytes was measured as 7.25±0.14µm. The average size values were calculated as 40±1.58µm^2. According to Silva et al. (2002), the diameter of prohemocytes in *Anastrepha obliqua* larvae was reported to be in the range of 7.5-13.12 µm. Prohemocytes are undifferentiated cells found in the hematopoietic system of insects, particularly in the larvae and pupae stages (Lanot et al., 2001). They are typically located in the hematopoietic organs of insects, such as the lymph glands (Lan et al., 2020). These cells are capable of differentiating into other types of

hemocytes (plasmatocytes or granulocytes), which play a crucial role in the immune response of insects (Yamashita and Iwabuchi, 2001). Wu et al. (2016) stated that prohemocytes were not detectable in the hemolymph of *Galleria mellonella* larvae since they quickly transformed into different types of hemocytes.

Plasmatocytes have a spherical shape and do not vary in form; they are usually found in groups within the hemolymph. The nucleus of plasmatocytes is also spherical and is typically located near the center of the cell. The average length values of plasmatocytes were measured as 10.50±0.74 μm. The average size values were calculated as 96±1.39 μm^2. In *A. obliqua* larvae, Silva et al. (2002) found that spherical plasmatocytes had a diameter of 13-26 μm, while oval plasmatocytes were 26-34 μm in length and 15-30 μm in width. Plasmatocytes, which are similar to vertebrate macrophages, play a role in phagocytosis, eliminating apoptotic cells during development, encapsulating or digesting pathogens (Evans et al., 2003; Hartenstein, 2006).

Prohemocytes and plasmatocytes are similar in shape. It was observed that prohemocytes were usually single, while plasmatocytes were usually composed of several cells. In prohemocytes, it was observed that the nucleus filled almost the entire cell and the cytoplasm occupied very little space. In plasmatocytes, the nucleus is centrally located and includes most of the cell. Plasmatocytes are generally spherical in shape, and cytoplasmic extensions are not observed.

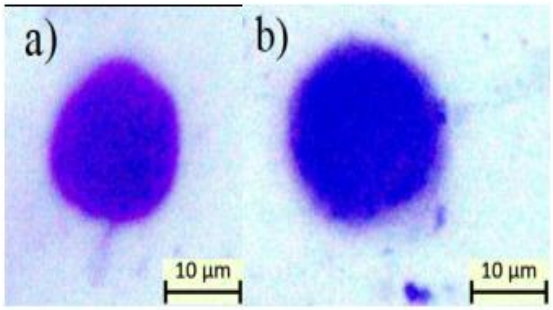

Figure 1. Hemocyte species detected in the hemolymph of *Lepisma saccharina* a) Prohemocyte b) Plasmatocyte

Previous studies on hemolymph in *Lepisma saccharina* primarily focused on amino acid determinations (Punzo, 1987). *Lepisma saccharina* species was included in the Zygentoma order instead of the Thysanura order. Gupta did not conduct a study specifically on this order because it was classified as a subclass within the Thysanura order until a new classification was made as a separate order. Gupta (1979) included granulocyte, plasmatocyte and spherulocyte types in the palaeoptera included in the Thysanura team. In the current study, prohemocyte and plasmatocyte types were observed in the hemolymph of samples belonging to *Lepisma saccharina* species, while granulocyte and spherulocyte types were not observed. Prohemocyte was detected in the samples in accordance with the studies where prohemocyte is considered as the common origin of hemocyte types, especially plasmatocyte. For example, in a study conducted in the larvae of *Lithacodes fasciola* species, hemolymph ranged between 1.10 × 106 - 8.23 × 106 cells/ml (Stoepler et al., 2012).

4. Conclusion

In the relatively primitive insect species, ametabolous Lepisma saccharina, two hemocyte types—prohemocytes and plasmatocytes—were identified. The absence of granulocytes, which play

significant roles in the immune system, suggests that *Lepisma saccharina* has a more primitive immune system. It can also be interpreted that the immune system they have in terms of the habitat is sufficient to contribute to the survival of the insect. Prohemocytes are smaller in size than other cells and similar in shape and are spherical. Only spherically shaped plasmatocytes were detected in *Lepisma saccharina*. Plasmatocytes were larger cells than prohemocytes and were usually seen in groups. Although the prohemocytes detected in the hemolymph of *Lepisma saccharina* species are smaller in size, they have a nucleus that fills almost the entire cell. It was determined that prohemocytes were smaller in size than other cells. In the studies conducted by Gupta (1979), it was determined that prohemocytes were generally 6-14 µm in diameter. In the current study, the mean diameter of prohemocytes in *Lepisma saccharina* was measured as 7.25 µm.

In the current study, plasmatocytes in *Lepisma saccharina* had a spherical shape and when examined morphologically, it was determined that they did not show diversity and were generally found in the hemolymph in groups. Besides, in plasmatocytes detected in this species, the nucleus has a spherical shape and is usually located close to the center. It was stated by Gupta (1979) that plasmatocytes could be 3.3-5 µm in width and 3.3-40 µm in length. In the current study, the mean diameter detected in spherically shaped plasmatocytes in *Lepisma saccharina* was measured as 10.50 µm. It can be said that the absence of granulocytes, which play an important role in the immune system, *in Lepisma saccharina* indicates that it has a more primitive immune system compared to other insects.

Based on the literature data, insects are incredibly diverse and have evolved a range of strategies for dealing with immune challenges. Therefore, hemocytes can vary in terms of their size, shape, and function, and different insect species may have different combinations of these cell types. Additionally, the density of hemocytes in the insect's hemolymph can also vary depending on a range of factors, including the insect's life stage, the type of infection or immune challenge it is facing, and the specific hemocyte types involved. Overall, the complex interplay between different types of hemocytes and their densities is a fascinating area of research that continues to yield insights into the remarkable immune defenses of insects.

In conclusion, insect immune system plays a pivotal role in shaping the evolution of insect-pathogen interactions, with pathogens evolving strategies to evade immune responses and insects developing countermeasures to detect and combat these pathogens. By understanding the complexity and adaptability of the insect immune system, we can gain insights into how insects have evolved to survive in their environments, which may inform new strategies for controlling insect-borne diseases.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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