Micron 43 (2012) 978–984

Contents lists available at SciVerse ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Ultrastructure of the spermatozoa of *Cicadella viridis* (Linnaeus) and its bearing on the phylogeny of Auchenorrhyncha

Beibei Zhang, Wu Dai*

Key Laboratory of Plant Protection Resources and Pest Integrated Management, Ministry of Education, Entomological Museum, Northwest A&F University, Yangling, Shaanxi 712100, China

ARTICLE INFO

Article history:
Received 28 February 2012
Received in revised form 27 March 2012
Accepted 27 March 2012

Keywords:
Spermatozoon
Cicadellidae
Centriolar adjunct
Accessory bodies
Morphology

ABSTRACT

The ultrastructure of mature spermatozoa of the leafhopper *Cicadella viridis* (Linnaeus) was investigated using light and transmission electron microscopy. The spermatozoon is composed of a head containing an acrosome and an elongated nucleus, and a long tail, which consists of a flagellum. The acrosome is conical and invaginated to form a subacrosomal space, and the acrosomal contents are filled with electron-dense tubular substructures. The nucleus is linear and filled with homogeneously condensed chromatin. The centriolar adjunct is parallel to the nucleus and connects the nucleus with the mid-piece/flagellum. The flagellum is formed by a 9+9+2 axoneme, two mitochondrial derivatives and two accessory bodies. The mitochondrial derivatives with an orderly array of peripheral cristae are symmetrical. The accessory bodies are small and slightly elliptical. The end of the axoneme shows progressive loss of microtubules. Comparison of sperm ultrastructure of *C. viridis* with those of other Auchenorrhyncha families supports the major relationships within Cicadomorpha as (Membracoidea (Cicadoidea, Cercopoidea)).

1. Introduction

Spermatozoa are highly specialized cells with many unique features. Sperm morphology can evolve rapidly (Baccetti and Afzelius, 1976; Jamieson, 1987; Joly et al., 1989) and displays spectacular diversity (Swallow and Wilkinson, 2002). Different forms of sperm amongst species are also associated with different reproductive strategies (Simmons, 2011) and variation in sperm morphology provides a useful tool for the reconstruction of phylogenies in various insect groups (Baccetti and Afzelius, 1976; Jamieson, 1987). Comparative studies on the spermatozoa of different groups can improve our understanding of specific morphological differences and provide a valuable theoretical basis for determining their taxonomic status and relationships (Franzén, 1970; Jamieson, 1987). Sperm structure can also help elucidate phylogenies, when combined with a corresponding current classification system (Baccetti, 1979; Jamieson, 1987). Recent studies have indicated that the structure and ultrastructure of insects' internal reproductive organs and spermatozoa provide additional characters for taxonomic analysis and can provide further understanding of insect relationships (Alves et al., 2006; Dallai et al., 2008; Araújo et al., 2009, 2010, 2011; Name et al., 2010; Xie and Hua, 2010; Simmons, 2011; Vitale et al., 2011). However, in such analyses it is necessary to examine sperm from a number of representative taxa before the usefulness of sperm morphology in phylogeny can be evaluated.

In the hemipteran suborder Auchernohyncha, studies on ultrastructure of spermatozoa have been conducted for a few species of Cicadellidae (4 species), Cicadidae (12 species), Aetalionidae (1 species), Cercopidae (2 species) and Fulgoromorpha (2 species) (Folliot and Maillet, 1970; Dai et al., 1996; Kubo-Irie et al., 2003; Chawanji et al., 2005, 2006; Araújo et al., 2010). Leafhoppers (Cicadellidae), a diverse group of Auchenorrhyncha with over 20,000 species described in 1500 genera and cosmopolitan in distribution, comprise by far the largest family within the Hemiptera. Apart from causing direct damage by their feeding and ovipositing activities, more than 100 species vector pathogens causing serious plant diseases (Dietrich, 2002).

To date, the spermatozoa of only one leafhopper species, *Dalbulus maidis* (Delong and Wolcott), have been described in detail (Cruz-Landim and Kitajima, 1972) and three species (*Cicadella viridis* (Linnaeus, 1758), *Ulopa reticulata* (Fabricius, 1794) and *Typhlocyba douglasi* (Edwards, 1878)) have been briefly mentioned. *C. viridis* is a large, widespread and conspicuous species common throughout the Palaearctic region (Wilson et al., 2009). Here we describe in detail the morphology and ultrastructure of the spermatozoa of *C. viridis* as an example of leafhoppers that may be useful for comparison with other species from the family Cicadellidae, and provide some foundations for future taxonomic and phylogenetic analyses.

* Corresponding author.
E-mail address: daiwu@nwsuaf.edu.cn (W. Dai).

0968-4328/$ – see front matter.
http://dx.doi.org/10.1016/j.micron.2012.03.022

2. Materials and methods

The insects studied were adult males of the phytophagous leafhopper *C. viridis* (Hemiptera, Cicadellidae), obtained from grass on the farms of Northwest A&F University, Shaanxi Province, China.

2.1. Light microscopy

Five male adult *C. viridis* were collected by aspiration from a gauze cage and anaesthetized with a slight stream of carbon dioxide. Dissections were performed in physiological saline solution under a Nikon SMZ1500 stereo microscope. Spermatozoa were obtained from the testes and seminal vesicles. Sperm samples were spread evenly on a microscope slide covered with the cover slips. After being allowed to dry for 1 min at room temperature, sperm were examined and photographed using Nikon Eclipse 80i microscope.

2.2. Transmission electron microscopy (TEM)

After live male adults were anesthetized in isoamyl acetate, their testes and seminal vesicles were dissected out rapidly in Ringer's solution (NaCl:CaCl:KCl:dH_2O = 10 g:0.34 g:0.3 g:1100 ml) and then fixed in 2.5% glutaraldehyde in phosphate buffer saline (PBS, 0.1 M, pH 7.2) for 12 h. The fixed specimens were kept separately with a small ice block in a Styrofoam box before being stored at 4 °C.

The fixed specimens were rinsed with 0.1 M phosphate buffer solution (PBS), and post-fixation was performed in 1% osmium tetroxide for 2 h at 4 °C. After four rinses (15 min per rinse) in the same buffer, the samples were dehydrated through a graded series of ethanol and embedded in Epon 812. Ultrathin sections were cut using a diamond knife on an ultramicrotome (Reichert Ultracut E), stained with uranyl acetate and lead citrate. Sections were examined and photographed with a JEOL JEM-1230 transmission electron microscope at 80 kV.

3. Results

The spermatozoa of *C. viridis* are organized into bundles, and their anterior ends are embedded in a homogenous matrix forming a spermatodesm (Figs. 1A and D, 2D and F).

The spermatozoa of *C. viridis* are all about the same length, not polymorphic (Fig. 1B). The mature spermatozoon is elongated and filiform, measuring approximately 135 μm in total length, composed of a slender, needle-like head (≈30 μm in length) and a long tail or flagellum (≈105 μm in length), which attaches to the head by the centriolar adjunct, in the nucleus-flagellum transition region (Figs. 1C and 2A).

The head consists of a short acrosome (≈3.4 μm in length) and an elongated nucleus (Fig. 2F). The acrosome is conical and deeply invaginated posteriorly to form a subacrosomal space (Fig. 2D and G). Anteriorly the subacrosomal space is eccentric in position (Fig. 2D and G). The acrosomal contents are not homogenous in appearance but are filled with electron-dense tubular substructures. The subacrosomal space and the tubular substructure are clearly visible in cross-section (Fig. 3A–C). In the middle of the acrosome, there is a distinct electron-lucent area (Fig. 2D). The acrosome is tapered anteriorly, posteriorly it forms tubular acrosomal processes that flank the nucleus anteriorly (Figs. 2G, 3D and E).

The nucleus is linear, long, and filled with homogeneously condensed chromatin. As shown by the cross-section view, the shape of the nucleus varies from deltoid to oval (Figs. 1D and 3F). Its base is laterally flattened at the nucleus-flagellum transition region. In this region, on one side, the nucleus extends together with the centriolar adjunct and then with the mitochondrial derivatives (Fig. 3H). The centriolar adjunct can be regarded as the start of the middle region and lies parallel to the nucleus (Figs. 2B and 3G). The centriolar adjunct, a lamellate structure, is relatively short, positioned to one side of the nucleus (Fig. 2B). On the other side, the flatter nucleus ends above the centriole region, which connects the nucleus with the axoneme (Figs. 2B and C and 3I).

The flagellum consists of an axoneme, two accessory bodies and two mitochondrial derivatives. The axoneme displays the typical insect pattern of 9 + 9 + 2 microtubules being 9 accessories, 9 doublets and 2 central microtubules, where the nine single accessory microtubules are the most external followed internally by the nine doublets and a central pair (Fig. 4C and D). The accessory microtubules have coarse fibers between each other, and the double microtubules have dynein arms and thick radial spokes directed towards the central complex (Fig. 4C). At the posterior extremity, the axoneme becomes gradually disorganized, the final portions of the flagellum are the nine doublet microtubules, which demonstrates that the doublet microtubules are the last to terminate whilst the other two microtubules structures disappear first (Fig. 4G and H).

Mitochondrial derivatives are symmetrical and extend along most of the length of the flagellum, positioned lateral to the axoneme and begin adjacent to the posterior extremity of the centriolar adjunct (Fig. 2C). The mitochondrial derivatives are rabbit ear-shaped in cross-section and each derivative has two different regions: an electron lucent region and an electron-dense region (Fig. 4D and E). In longitudinal sections, the peripheral cristae are perpendicular to the axis of the derivatives and spaced at regular intervals measuring 12 nm (Fig. 2E).

The accessory bodies are small and circular or orbicularovate in cross-section. They lie between the axoneme and the mitochondrial derivatives, adjacent to the axoneme, parallel to the mitochondrial derivatives. However, the accessory bodies do not extend the same length compared with the axoneme and the mitochondrial derivatives, as shown by the cross-sections (Fig. 4A, B, D and E). In addition, during the elongation process of the spermatid, the accessory bodies are the first to complete the process (Fig. 4D and E), the mitochondrial derivatives then disappear and, finally, the structure of the axoneme becomes irregular (Fig. 4F).

4. Discussion

In the suborder Auchenorrhyncha, ultrastructure of spermatozoa of the Cicadomorpha (Cicadidae, Cercopidae, Aethalionidae and Cicadellidae) and Fulgoromorpha infraorders have been described, but for very few species (Folliot and Maillet, 1970; Kubo-Irie et al., 2003; Chawanji et al., 2005, 2006; Araújo et al., 2010). This study represents the most detailed description of the sperm ultrastructure of a leafhopper (Cicadellidae). Previous authors provided general descriptions of leafhopper sperm that lacked critical details (Folliot and Maillet, 1970; Cruz-Landim and Kitajima, 1972).

The spermatozoa of *C. viridis* (Cicadellidae) have the following morphological similarities to the spermatozoa of the other families of Auchenorrhyncha so far studied: (1) spermatozoa aggregate in a homogeneous matrix forming a spermatodesm; (2) a conical acrosome with electron-dense tubular substructures, which forms a subacrosomal space; (3) absence of a perforatorium; (4) the base of the nucleus with condensed chromatin is laterally pointed forming a nucleus-flagellum transition region; (5) a centriolar adjunct parallel to the nucleus that terminates in a region where the mitochondrial derivatives start; (6) two mitochondrial derivatives located laterally and extending along the axoneme; (7) a single axoneme with a typical 9 + 9 + 2 pattern of microtubule arrangement.