REDIA, XCII, 2009: 195-197

DANILO G.M. VITALE (*) - MARIA VIOLETTA BRUNDO (*) - LORENZO SOTTILE (*)
RENATA VISCUSO (*) - SEBASTIANO BARBAGALLO (**)

MORPHOLOGICAL AND ULTRASTRUCTURAL INVESTIGATIONS OF THE MALE REPRODUCTIVE SYSTEM IN APHIDS: OBSERVATIONS ON *TUBERCULATUS (TUBERCULOIDES) EGGLERI* BÖRNER (HEMIPTERA APHIDOIDEA)

(*) *Dipartimento di Biologia Animale "M. La Greca", Università degli Studi, via Androne 81, 95124 Catania, Italy;*
viscure@unict.it
(**) *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, via S. Sofia 100, 95123 Catania, Italy.*

Vitale D.G.M., Brundo M.V., Sottile L., Viscuso R., Barbagallo S. – Morphological and ultrastructural investigations of the male reproductive system in aphids: observations on *Tuberculatus (Tuberculoides) eggleri* Börner (Hemiptera Aphidoidea).

Investigations into the amphigonic reproductive mechanisms of species belonging to the Superfamily Aphidoidea are likely provide useful information on aphid reproductive biology. Therefore, a preliminary ultrastructural study of their reproductive system, of which almost nothing is recorded in the literature, could be opportune.

Our objective was to increase our knowledge of the male reproductive system in aphids by studying the ultrastructure of the male genitalia of several species.

In this study, *Tuberculatus (Tuberculoides) eggleri* Börner (Aphididae, Calaphidinae) is used to describe the general characteristics of the male reproductive system in aphids.

KEY WORDS: Aphididae; male reproductive system; ultrastructure; spermatodesms.

INTRODUCTION

Because of their peculiar structural, biological and reproductive characteristics, such as the well-known alternation of parthenogenesis and amphigony, insects belonging to the Superfamily Aphidoidea deserve special attention. Amphigonic individuals occur in aphid populations during a period of approximately one-month, between autumn and winter, which is related to the close association between aphids and their host plants.

A fertilized egg invariably produces a female ("fundatrix") because of the peculiar characteristics of aphid spermatogenesis in which gametes with an A0 chromosomal pattern degenerate and only the AX gametes survive (HONDA, 1921). A survey of these processes in aphids will undoubtedly provide useful information on their reproductive biology. However, a preliminary ultrastructural study of the male reproductive tract of these insects is required as there is almost nothing in the literature on this subject.

In order to achieve this, the morphology and ultrastructure of the testes and spermducts of several species of Aphididae were studied. Despite the difficulty of finding amphigonic individuals in any aphid population in Sicily because of the mild Sicilian weather and the consequent reduction or total absence of the period when sexuals are produced, a sufficient number of sexually mature males were collected.

In this study, *Tuberculatus (Tuberculoides) eggleri* Börner (Subfamily Calaphidinae) is used to describe the general characteristics of the male reproductive system in aphids.

MATERIALS AND METHODS

Sexually mature males of *T. eggleri* were collected at Aci S. Antonio (Catania), located 250 m a.s.l., between mid-January to mid-February. The insects were kept alive by keeping them at 4 ± 1°C and used within two weeks of collection.

For *in toto* observation of the genital tracts under a stereomicroscope individuals were dissected in Ringer solution. For transmission electron microscope (TEM, Philips CM10) genital tracts were fixed in 2.5% glutaraldehyde in 0.1M Sorensen buffer (SB) at pH 7.4 for 4 hours at room temperature (r.t.). After washing in SB and post-fixing in 1% osmium tetroxide in SB for 1 h. at r.t., samples were dehydrated in a graded ethanol series, immersed in propylene oxide and embedded in Embed 812 (TAAB). Ultrathin sections, 40-70 nm, were mounted on copper-rhodium grids (200/300 mesh) and stained with uranyl acetate and lead citrate.

The histological investigations were carried using an optical microscope (OM) and semithin sections, 400-700 nm, mounted on microscope slides, the resin removed by placing in a saturated solution of sodium hydroxide in absolute ethanol and stained with 0.5 % Toluidine blue in SB.

For scanning electron microscopy (SEM, Cambridge Stereoscan 360) genital tracts were fixed in 2.5% glutaraldehyde in 0.1M SB, for 1h. at r.t.. After washing in SB, samples were dehydrated in a graded ethanol series, immersed in hexamethyldisilazane (HMDS) and air dried at r.t.; finally, the genital tracts were mounted on SEM stubs and coated with metal.

– Received 30 July 2009 Accepted 2 September 2009 Published 1 December 2009

RESULTS AND DISCUSSION

Overall Morphology

The general anatomical and morphological characteristics are similar to those described in the literature (Wieczorek, 2006) (Fig. I, 1). The testes are located dorsally above the gut and appear to make up a single body; each testis consists of 3 large ellipsoidal follicles opening separately into the corresponding spermduct. The diameter of the two spermducts is uniform except for the slight enlargement proximal to the testis; at the tip of this enlargement the walls of the two spermducts adhere together. Each spermduct runs rectilinearly and opens, below the gut, into a reduced ejaculatory duct that terminates at the gonopore. Two club-shaped accessory glands open into the median portion of the ejaculatory duct.

Although already described in the literature on aphids and other insects it is important to stress that the seminal vesicles are not morphologically distinct from the spermduct. However, it is possible that the enlargement of the spermduct also functions as a seminal vesicle (Blackman, 1987).

Histology and fine structure

The histological and ultrastructural organization of the male reproductive tract is very simple and resembles that described by Wieczorek & Swiatek (2008). Each follicle contains several cysts arranged in order of increasing maturation, from the distal to the proximal tract; gametes, within every cyst, are at the same stage of maturation. However, meiotic processes were not observed, since these processes generally only occur in the embryonic and nymphal stages of male aphids (Blackman, 1987).

In the more proximal cysts, mature spermatids are aligned in bundles (Fig. I, 2) corresponding to the spermatodesms in other species of insects, such as Orthoptera (Viscuso *et al.*, 1998). In each bundle, gametes seem to be kept together by a loosely structured "cap" (Fig. I, 2, c).

Our results seem to indicate that this cap is derived from material released during spermatid maturation and cyst-wall cell degeneration. This hypothesis arises from the TEM observation that there are numerous tubular structures, with a diameter of about 25 nm (Fig. I, 2, c, arrow), and tiny granulations in the cap resembling, respectively, cytoplasmic microtubules and ribosomes. It is worth noting in this regard, however, that even in the testicular lumen of the lepidopteran *Phragmatobia fuliginosa* (Wolf, 1993), there are tubular structures of probable cytoplasmic derivation.

The TEM observations indicate there are flattened cells in the walls of the testicular cysts that have large and irregular nuclei elongated along the major axis of the cell. Moreover, the cell cytoplasm in the proximal cysts contains few organules and numerous lamellar bodies. These characteristics, as in most insects, are probably associated with cyst degeneration.

The ultrastructural characteristics of the spermduct (Fig. I, 3) are similar to those of most insects, it consisting of a muscle-connective sheath surrounding a mono-

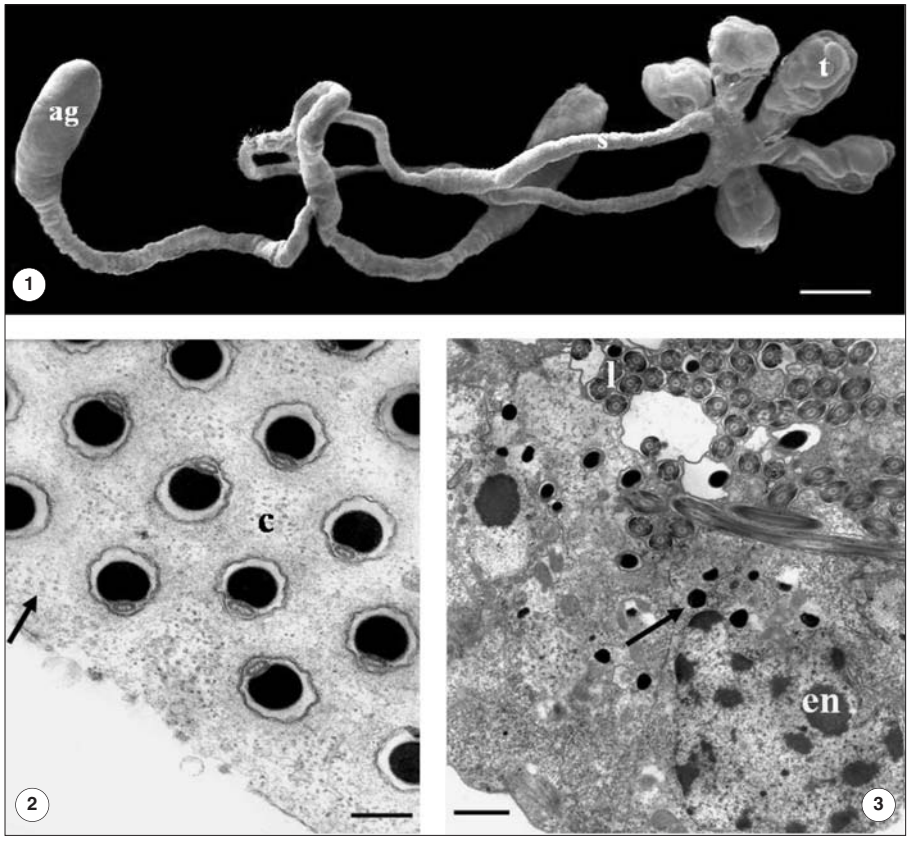

Figure I

1, male reproductive system. SEM. ag, accessory glands; s, spermduct; t, testis. Bar = 100 μm. 2, cross section through the nuclei of a sperm bundle in a proximal testicular cyst; c, cap interposed between gametes, with tubular structures (arrow) and granules. TEM. Bar = 350 nm. 3, cross section of a spermduct. The epithelial cells in the spermduct wall have large nuclei (en) and characteristic intracytoplasmic granules (arrow). The spermduct lumen (l) is filled with sperm. TEM. Bar = 1 μm.

stratified epithelium, devoid of a cuticle and lying on a basal lamina. The epithelial cells display lateral membrane interdigitations with extensive septate junction systems.

However, the most peculiar characteristic of the sperm-duct of *T. eggleri* is the presence of vesicles, with a heterogeneous content of different electrondensity, in the cytoplasm of the epithelial cells involved in secretion (Fig. I, 3, arrow).

These results constitute a promising start to a study of the role of aphid male genital structures in reproduction. In addition, comparative ultrasructural studies of the reproductive system of aphids are likely to provide information of systematic importance .

ACKNOWLEDGEMENTS

This research was supported by a MIUR grant funding the study of: “Structural and molecular studies on Insect reproduction”.

REFERENCES

BLACKMAN R.L., 1987 – *Reproduction, cytogenetics and development.* In: Aphids. Their Biology, Natural Enemies and Control. Minks. A.K. and Harrewijn P. eds. Elsevier, Amsterdam, vol. 2A: 163-195.

HONDA H., 1921 – *Spermatogenesis of aphids: the fate of the smaller secondary spermatocyte.* - Biological Bullettin of the Marine Biology Laboratory, Woods Hole, Mass, 40: 349-369.

VISCUSO R., NARCISI L., SOTTILE L., BARONE N., 1998 – *Structure of spermatodesm of Orthoptera Tettigonioidea.* - Tissue and Cell, 30(4): 453-463.

WIECZOREK K., 2006 – *Anatomical investigations of the male reproductive system of five species of Calaphidinae (Hemiptera: Aphidoidea).* - Insect Syst. Evol., 37: 457-465.

WIECZOREK K., SWIATEK P., 2008 – *Morphology and ultrastructure of the male reproductive system of the woolly beech aphid* Phyllaphis fagi *(Hemiptera: Aphididae: Phyllaphidinae).* - Eur. J. Ent., 105: 707-712.

WOLF K.W., 1993 – *Extracellular tubular elements within testes of* Phragmatobia fuliginosa *L. (Arctiidae, Lepidoptera).* - Inv. Rep. and Dev., 24 (2): 137-142.

Bianca