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RELEASE, BEHAVIOR AND PHYLOGENETIC SIGNIFICANCE OF SPERMATOZOA IN BUNDLES IN THE SEMINAL VESICLE DURING SEXUAL MATURATION IN ACULEATA (HYMENOPTERA)

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ABSTRACT

In this report, we describe how spermatozoa are released from the testis in the Aculeata *Melipona quadrifasciata* (Apidae) and *Acromyrmex subterraneus* (Formicidae). Immature and sexually mature adult males of both species were dissected and their seminal vesicles, vas deferens and testes were processed for light and transmission electron microscopy. At the end of spermiogenesis, the spermatozoa still formed bundles, with the anterior end of their heads inserted into electron dense material. These bundles migrated to the seminal vesicles and, as the adults matured sexually, the bundles started to disorganize and the spermatozoa became separated. At the end of sexual maturation, the seminal vesicles contained no bundles, but were completely full of free spermatozoa. In the Symphyta, as in the Aculeata studied here, the spermatozoa leave the testis in bundles. But there is no dissociation of the spermatozoa from the bundles during sexual maturation. The Aculeata have been suggested to be derived from parasitic wasps that, in turn, derived from the Symphyta. Hence, the release of spermatozoa in bundles in the Aculeata is a reverse characteristic or may indicate that this group is derived directly from the Symphyta.

Key words: Aculeata, phylogeny, spermatodesmata, spermatozoa, sperm bundles

INTRODUCTION

The Hymenoptera (ants, bees and wasps) is one of the four largest orders of insects, with about 115,000 known species, and is traditionally divided in two suborders, Symphyta and Apocrita. The Apocrita contains most of the known species and is divided into Aculeata and Parasitica. The Aculeata is considered to be a monophyletic group and is the most derived [3]. Despite of economic and ecological importance of this group [2], its evolutionary history is still unclear.

Spermatozoan morphology has provided information about the phylogenetic relationships of many insect groups, including the Hymenoptera [4]. However, much information can still be obtained by studying the behavior of these cells in the male reproductive system.

In insects, spermatozoa can be transferred from males to females in a spermatophore (as in most Lepidoptera), in spermatodesmata, or individually. The last two mechanisms are usually found in the Symphyta and parasitic wasps, respectively. Spermatodesmata are groups of spermatozoa connected by their heads by an extracellular matrix [12,14]. This glycoprotein matrix is secreted by cystic cells and become associated with the anterior region of the spermatozoan head at the end of spermiogenesis. This association keeps the spermatozoa together during their transference to the seminal vesicles. Bundles of spermatozoa have been found in the seminal vesicles of all Symphyta examined so far [11,12,14]. In contrast, the occurrence of this characteristic in the Aculeata is unclear, although some bundles or bundle fragments have been observed in the seminal vesicles of bees of the tribe Apini [1,16,17] and in the Euglossinae [18]. In parasitic wasps, only free spermatozoa have been observed [5-12]. In this report, we describe the behavior of the spermatozoa along the male genital tract in two species of Aculeata and discuss its importance as a phylogenetic indicator in Hymenoptera.

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MATERIAL AND METHODS

Insects

Immature and sexually mature adult males of *Melipona quadrifasciata* and *Acromyrmex subterraneus* were obtained

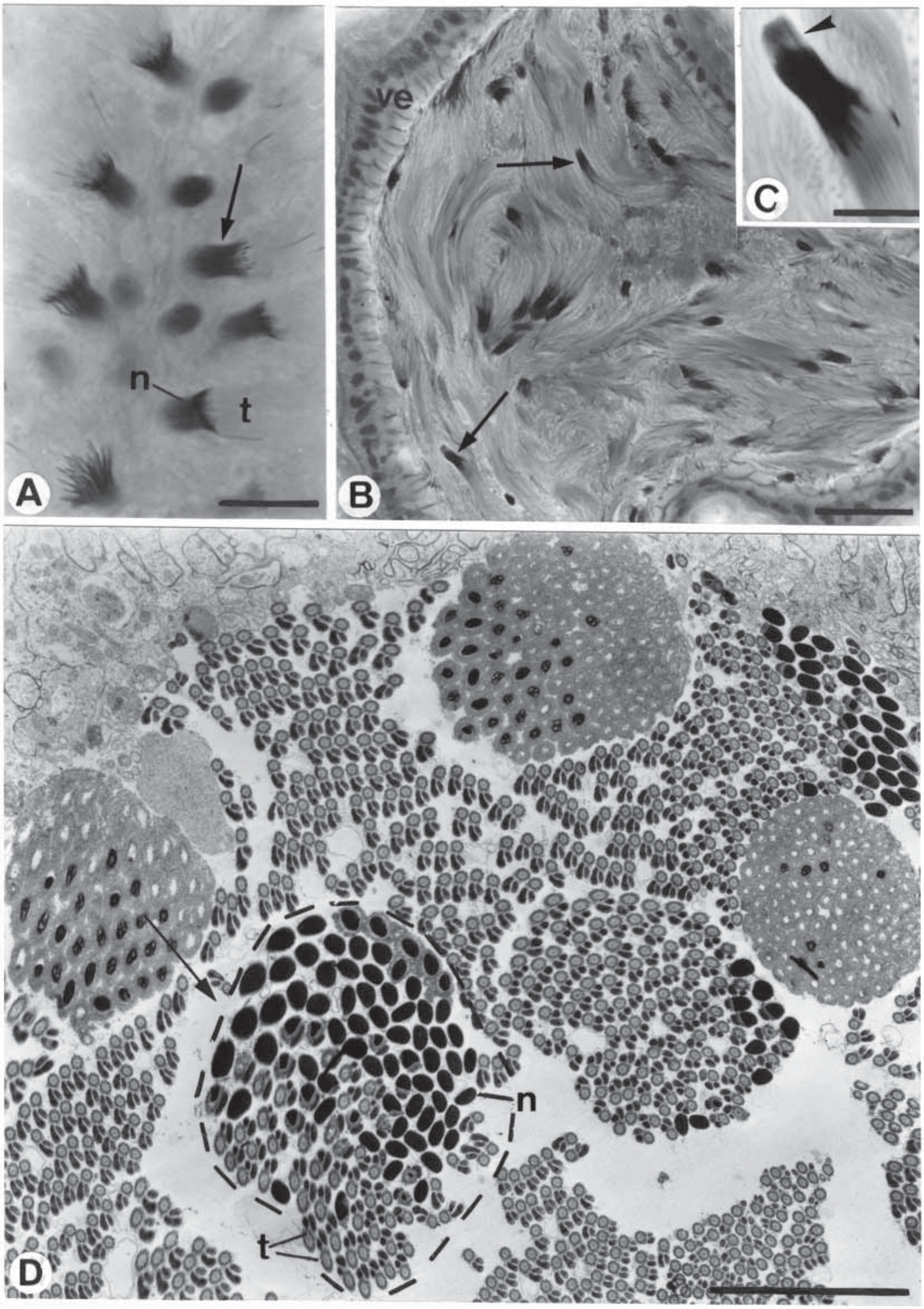

Figure 1. Light micrographs (**A-C**) and transmission electron micrograph (**D**) of the testis (**A**) and seminal vesicle (**B-D**) of an immature male of *Melipona quadrifasciata*. Note the spermatozoa organized in bundles in the testis (**A**) and seminal vesicle (**B-D**). The arrows indicate the bundles of spermatozoa and the arrowhead, the glycoprotein located at anterior end of the spermatozoa. **Dashed line** – bundle of spermatozoa, **n** – spermatozoa nucleus, **t** – tail, **ve** – vesicular epithelium. Bars: A = 20 µm, B = 50 µm, C = 10 µm and D = 5 µm.

from nests kept in the central apiary and in the insectary, respectively, at the Federal University, at Viçosa.

Light microscopy

The testis, seminal vesicles and vas deferens were dissected and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2, and embedded in Historesin®. Semithin sections were stained with toluidine blue and photographed.

Transmission electron microscopy

The seminal vesicles were dissected and fixed for 3 h in a solution containing 2.5% glutaraldehyde, 0.2% picric acid, 3% sucrose and 5 mM $CaCl_2$ in 0.1 M cacodylate buffer, pH 7.2. After rinsing in buffer, the material was post-fixed in 1% $0sO_4$, dehydrated in acetone and embedded in Epon 812 resin. Ultrathin sections doube-stained with uranyl acetate and lead citrate were observed in a Zeiss Leo 906 transmission electron microscope.

RESULTS

In males of both species, the spermatids developed in cysts in the testicular follicles during spermiogenesis. At the end of this process, the spermatozoa were arranged in bundles with the nuclei oriented in the same direction (Fig. 1A). An electron dense material was produced at the end of spermiogenesis and became associated with the anterior region of the spermatozoan heads (Fig. 1C). This material was PAS-positive, indicating it was glycoprotein.

At the end of spermiogenesis, the cysts opened but the spermatozoa were kept in bundles by the glycoprotein. These bundles were transferred through the vas deferens (Fig. 3A) to the seminal vesicles (Figs.1B and D, 2A and 3B). As the males became sexually mature, the spermatozoa were released from the bundles and, when totally mature, only free spermatozoa were see completely occupying the seminal vesicles (Figs. 2A and 3C).

DISCUSSION

Although the occurrence of spermatozoa in bundles has been observed in the seminal vesicles of some Aculeata [16-18], the behavior of these bundles in the seminal vesicle is still poorly understood. The organization of spermatodesmata has been reported in details for the Symphyta [14], with the spermatozoa remaining in bundles until copulation. However, as shown here, the release of spermatozoa in bundles from the testis also occurs in the Apocrita, specifically in the Aculeata, Apidae and Formicidae. In contrast to the Symphyta, the bundles disorganize during sexual maturation. We have observed the same behavior in the Sphecidae, although in parasitic wasps there was no bundle in the seminal vesicles [5-12].

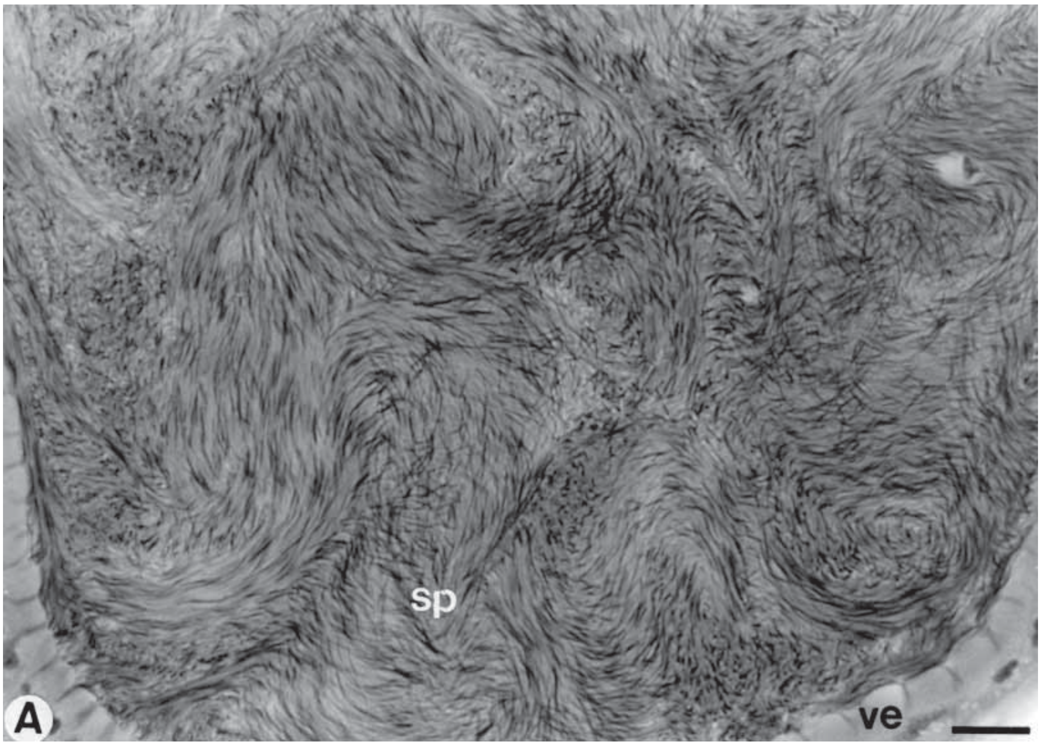

Figure 2. Light micrograph of the seminal vesicle (**A**) of mature males of *Melipona quadrifasciata*. Note free spermatozoa (**sp**) in this phase. **ve** – vesicular epithelium. Bar = 50 µm.

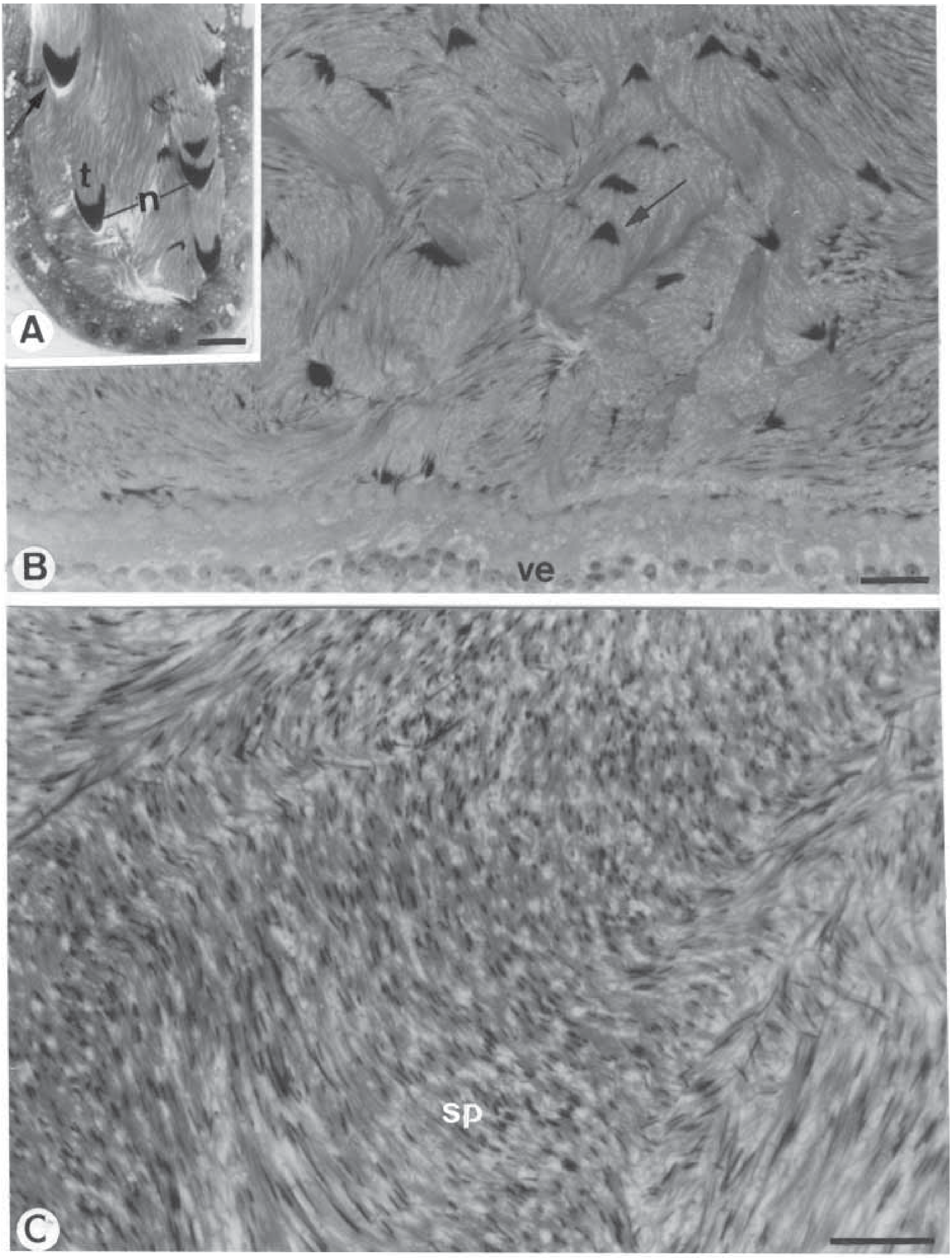

Figure 3. Light micrographs of the deferent duct (**A**) and seminal vesicles of immature (**B**) and mature (**C**) males of *Acromyrmex subterraneus.* Note the bundles of spermatozoa in the deferent duct (**A**) being transferred to the seminal vesicle (**B**). This bundle will become disorganized as the individual matures sexually (**C**). **Arrows** – bundles of spermatozoa, **n** – spermatozoa nucleus, **t** – tail, **ve** – vesicular epithelium, **sp** – free spermatozoa. Bars: A = 20 µm, B and C = 50 µm.

The organization of the spermatodesmata in the Aculeata is morphologically similar that observed in Symphyta [11,12,14,16-18]. Studies using molecular data and traditional morphological characters have suggested that the Aculeata derived from a parasitic ancester, which, in turn, derived from Symphyta [13,15]. Since spermatodesmata have not been observed in parasitic wasps [5-8], the presence of this characteristic in the Aculeata would necessarily be reverse, if the evolutionary history proposed by the foregoing studies is correct. Alternatively, the presence of spermatodesmata in the Aculeata and Symphyta may be a homology, with each deriving independently of the other and not from parasitic wasps.

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