

Comparative morphology of spermathecae in solitary and primitively eusocial bees (Hymenoptera; Apoidea)

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Abstract: Spermathecae of solitary and primitively eusocial bees from five major families were compared according to their chamber size, epithelial cell height (wall thickness), number and shape of glands, number of sperm pumps, and diameter of the duct, using whole mounts and serial sections. A thick spermathecal wall with a small chamber in our exemplar colletid, megachilid, and andrenid bees, a large chamber with a thick wall in social Apidae, and a thin wall with a large chamber in social halictids indicate that a big chamber may be associated with increased colony size. A thin epithelium in the receptacle of halictid bees suggests relegation of spermathecal secretion to the spermathecal gland. A relatively thick spermathecal wall in the Apinae indicates the potential importance of both the epithelial cell layer of the receptacle and the enlarged spermathecal gland in maintaining large numbers of stored sperm. There was one sperm pump in all taxa surveyed except the representative Halictidae, in which this structure was paired. Elongated spermathecal glands may typify the spermathecae of eusocial bees.

Résumé : La spermathèque a été étudiée par examen de préparations entières et de coupes sériées chez des guêpes solitaires et des guêpes eusociales primitives appartenant à cinq grandes familles : la taille de la chambre, la hauteur des cellules épithéliales (épaisseur de la paroi), le nombre et la forme des glandes, le nombre de pompes spermatiques et le diamètre du canal ont été comparés chez ces insectes. Une paroi épaisse avec petite chambre chez les collétides, mégachilides et andrénides, une grande chambre avec paroi épaisse chez les apides sociales et une paroi mince avec grande chambre chez les guêpes halictides sociales semblent indiquer qu'une chambre de grande taille est associée aux colonies de grande taille. L'épithélium mince du réceptacle des halictides semble indiquer que les sécrétions de la spermathèque proviennent exclusivement de la glande de la spermathèque. La paroi relativement épaisse de la spermathèque des Apinae indique que la couche de cellules épithéliales du réceptacle et la glande amplifiée de la spermathèque concourent probablement toutes deux à la mise en réserve de grands nombres de spermatozoïdes. Il n'y a qu'une seule pompe spermatique chez tous les taxons examinés, à l'exception du représentant des Halictidae qui compte une paire de pompes. Les glandes allongées de la spermathèque chez les guêpes eusociales sont probablement un caractère typique. [Traduit par la Rédaction]

Introduction

Following copulation, females of most insects store spermatozoa in the spermathecae, where they remain until they are used for fertilising eggs. Extensive studies of the spermatheca and its secretions in nonsocial species have explored the mechanisms that keep stored spermatozoa viable during the reproductive life of the female (Davey and Webster 1967; Clements and Potter 1967; Tombes and Roppel 1971, 1972; Villavaso 1975; Ahmed and Gillott 1982). Spermathecae provide both a suitable environment for the spermatozoa and a source of metabolites for their maintenance, the latter derived from secretions of glandular cells that either line the chamber or are separately located (Davey 1985; Gillott 1988; Kaulenas 1992). In the Hymenoptera, there is particularly wide variation in both the number of eggs laid and the length of time over which they are produced, giving rise to widely differing requirements in terms of numbers of

spermatozoa and the duration of their storage by females. Bees, in particular, display a wide array of social behaviours and range from solitary to eusocial forms, with multiple independent origins of a reproductive division of labour (Wilson 1971; Michener 1974). This unique situation lends itself to the study of reproductive mechanisms that accompany the evolution of social behaviour in this group.

Females of the solitary mining bee, *Andrena erythronii*, and the alfalfa leaf-cutter bee, *Megachile rotundata*, lay an average of approximately 8 and 20 diploid eggs, respectively, in their lifetime (Michener and Rettenmeyer 1956; Gerber and Klostermeyer 1970). Foundresses of the primitively eusocial *Lasioglossum laevisimum* produce a maximum of 52 worker-brood individuals (in a multiple-foundress nest) and 66 reproductives (Packer 1992), giving a total of approximately 75 diploid eggs requiring sperm for fertilisation. These are comparatively low reproductive outputs requiring fewer stored spermatozoa than, for example, in the perennial colonies of the primitively eusocial halictine bee *Lasioglossum marginatum*, where a single queen may need to fertilise over 2000 eggs (Plateaux-Quénu 1960). Spermathecae of *Apis mellifera* queens store sufficient spermatozoa to last their lifetime, during which hundreds of thousands of fertilised eggs are laid (Bresslau 1906; Snodgrass 1956; Poole 1970;

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Table 1. Summary of features and dimensions of the spermathecal complex of bees exhibiting various social behaviours.

	Behaviour ^a	Diapause between mating and oviposition?	N	Chamber diam., μm (A)		Epithelial cell height, μm (B)		Receptacle diam., μm (A + 2B)	No. of sperm pumps	Shape of glands ^b
				Mean	SD	Mean	SD			
<i>Leioproctus</i> spp.	SOL	No	5	39	0.007	41	0.005	121	1	S
<i>Megachile relativa</i>	SOL	No	42	84	0.023	41	0.007	166	1	S
<i>Andrena wilkella</i>	SOL	No	20	101	0.016	46	0.005	193	1	S
<i>Augochlora pura</i>	SOL	Yes	4	75	0.020	7	0.002	89	2	S
<i>Halictus ligatus</i>	PEU	Yes	47	122	0.011	7	0.002	136	2	E
<i>Lasioglossum marginatum</i>	Annual									
	PEU	Yes	29	219	0.034	3	0.001	225	2	E
<i>Bombus fervidus</i>	Perennial									
	PEU	Yes	21	172	0.017	26	0.002	224	—	E ^c
<i>Apis mellifera</i> ^d	Annual									
	HEU	No	—	920		40		1000	—	F ^c

^aSOL, Solitary; PEU, primitively eusocial; HEU, highly eusocial.

^bS, spheroid; E, elongated; F, filiform.

^cGlands are paired. Bresslau (1906) maintained that two muscle complexes make up the sperm pump in *A. mellifera*. Dallai (1975) observed six muscle groups constituting the sperm pump in the honey bee.

^dData are from Poole (1970) and Dallai (1975).

Dallai 1975, Winston 1987). Such large variation in egg production should be reflected in variation in spermathecal morphology.

In addition to the number of sperm required, the duration of their storage also varies greatly among bees. Some solitary bees lay their eggs soon after mating, so sperm are stored for only a short period. In contrast, all social and almost all solitary halictids diapause between mating and oviposition, requiring sperm to be stored for almost 1 year. Queens of perennially eusocial species have to store sperm for several years at least.

Sperm in the spermatheca must be conveyed to the site of fertilisation of the eggs; this is normally accomplished by peristaltic contractions of the spermathecal duct muscles (Bresslau 1906; Snodgrass 1956; Dade 1962; Ruttner and Koeniger 1971; Gessner and Ruttner 1977). The spermathecal gland muscles of *Megachile flavipes* have been reported to assist in conveying sperm through the ducts (Sihag 1986). In *Rhodnius prolixus*, contractions of the spermathecal muscles propel gland secretions from the glandular cells to the chamber of the spermatheca (Kuster and Davey 1983). Different requirements for sperm usage and storage may have selected for different designs of musculature associated with the spermatheca in bees.

This preliminary study was performed for three reasons: (i) to examine the possible relationship between the morphological and histological features of the spermathecal complex and the need for different numbers of sperm and different durations of storage, (ii) to determine which spermathecal features might be useful for more detailed analysis of the effect of social evolution upon spermathecal morphology, and (iii) to investigate the potential for spermathecal morphology to provide characters of systematic utility.

Materials and methods

Four solitary species, *Leioproctus* sp. (Colletidae), *Megachile relativa* (Megachilidae), *Andrena wilkella* (Andrenidae), and *Augo-*

chlora pura (Halictidae), and three primitively eusocial species, *Halictus ligatus* and *Lasioglossum marginatum* (Halictidae) and *Bombus fervidus* (Apidae), representing five major bee families were used in this study (see Table 1 for sample sizes for each species). Voucher specimens of all taxa surveyed are housed in the Canadian National Collection in Ottawa.

For light microscopy, spermathecae were excised from specimens preserved in buffered formalin. This was achieved by removing terga 2–5, locating the spermatheca amongst the other abdominal cavity structures, and excising the spermatheca and its associated duct by pulling the duct close to its point of juncture with the bursa copulatrix. Fine watchmaker's forceps were used throughout. Whole-mount preparations were either stained with Hansen's iron trioxynaematrin (Davey and Webster 1967) or carbol-thionine (Goukon et al. 1987) or left unstained. Other spermathecae were dehydrated and cleared in preparation for histological study. Paraffin blocks containing the spermathecae were serially sectioned and stained with Mallory's one step (Humason 1973). Spermathecal chamber size (diameter) and epithelial cell height or wall thickness (basement membrane to intima) of all the spermathecae were measured with an ocular micrometer.

Results

In the bees examined, the spermatheca is a spheroid storage organ connected by a duct to the bursa copulatrix, a wide membranous pouch at the anterior end of the sting chamber, which lies inside segment VI of the metasoma (Fig. 1). In all species, the spermatheca consists of four distinct parts: receptacle, gland, duct, and sperm pump. The receptacle is a chamber surrounded by epithelial cells. Depending upon the species, the gland is either apposed to both the receptacle and the duct or situated along one side of the duct opposite the receptacle. Similarly, the sperm pump is either housed in the gland, located along the spermathecal duct, or wedged between paired glands.

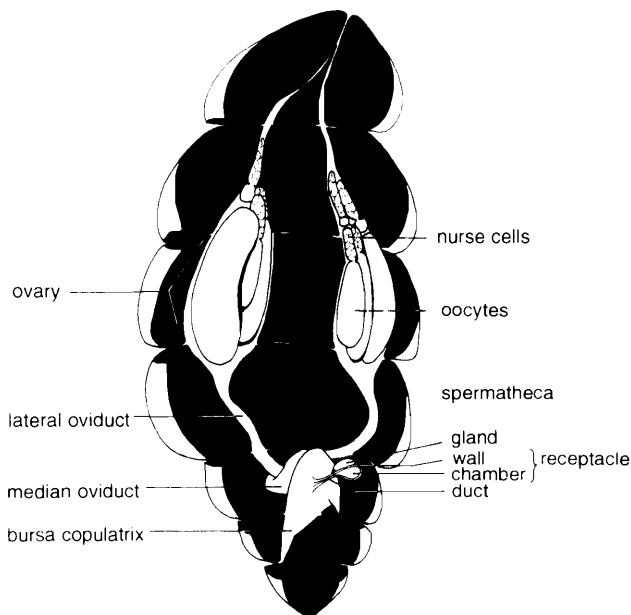
Spermathecal receptacle

From exterior to interior, the spermathecal receptacle consists of the epithelial cell layer (spermathecal wall), cuticular

ABBREVIATIONS: *ch*, chamber; *e*, epithelium; *c*, circular intima; *m*, sperm-pump muscles; *sd*, duct; *sg*, spermathecal gland; *sgd*, spermathecal gland duct; *sm*, sperm mass; *sp*, sperm pump; *sw*, spermathecal wall.

Fig. 2. Spermatheca of *Andrena wilkella*. (A) Whole mount. Unstained photomicrograph. (B) Diagram. **Fig. 3.** Spermatheca of *Halictus ligatus*. (A) Whole mount. Carbol-thionine stain. (B) Diagram. **Fig. 4.** Spermatheca of *Megachile relativa*. (A) Whole mount. Hansen's trioxyaematin. (B) Diagram. **Fig. 5.** Spermatheca of *Bombus fervidus*. (A) Whole mount. Unstained photomicrograph. (B) Diagram.

Fig. 1. The bee abdomen, showing the location of the spermatheca.



intima, and chamber (Fig. 2A). The chambers of four solitary species, *Leioproctus* sp. (Colletidae), *A. pura* (Halictidae), *A. wilkella* (Andrenidae), and *M. relativa* (Megachilidae), were considerably smaller in diameter than that of the annual primitively eusocial species, *H. ligatus* (Table 1). The chamber of *Bombus fervidus* (Apidae), another annual primitively eusocial species, had a larger mean diameter than that of *H. ligatus* but smaller than that of the perennial primitively eusocial *L. marginatum* (Halictidae). These data suggest that larger chambers typify the spermathecae of bees with larger colonies.

Columnar epithelial cells forming a single, possibly secretory layer in the spermathecal wall were most apparent in the solitary megachilid and andrenid bees (Figs. 2A, 4, and 6A). The representative species of Halictidae have thinner spermathecal walls (Figs. 3 and 6B, and Table 1) than bees of other families (Figs. 2, 4, and 5 and Table 1). The spermathecal wall of *L. marginatum* was the thinnest of all (Table 1) and its originally spheroid receptacle frequently became distorted (24 of 29) during preparation of whole mounts and serial sections. In contrast, none of the similar-sized receptacles was deformed during preparation of the spermathecae of *B. fervidus*, emphasizing the greater robustness of its wall (Table 1). The thickness of the cuticular intima was less than 1 μm in all the species examined.

Spermathecal gland

Spermathecal glands of all but two of the species examined abutted both the receptacle and proximal end of the duct

(Figs. 3–5). In *Leioproctus* sp. and *A. wilkella* the gland was situated along one side of the spermathecal duct opposite the receptacle (Fig. 2). *Leioproctus* sp., *M. relativa*, and *A. wilkella* each had one spheroid gland (Figs. 2 and 4). The solitary halictine, *A. pura*, had two spheroid glands, each surrounding a sperm pump, while the primitively eusocial halictines, *H. ligatus* and *L. marginatum*, had one elongate gland (Fig. 3). In *B. fervidus* the glands were paired, elongate, and separate from the sperm pump (Fig. 5), suggesting that elongate glands typify the spermathecae of eusocial species. The glandular cells surrounded the sperm pump in *M. relativa* (Fig. 4) and *A. pura*. The pump with its associated muscles was situated along the spermathecal duct, separate from the gland in *Leioproctus* sp. and *A. wilkella* (Fig. 2). The spermathecal glands were separate from the sperm pumps in *H. ligatus* (Fig. 3), *L. marginatum*, and *B. fervidus* (Fig. 5).

Spermathecal gland duct

A spermathecal gland duct was observed in species with the spermathecal gland separated from the sperm pump (*Leioproctus* sp., *A. wilkella*, *H. ligatus*, *L. marginatum*, and *B. fervidus*; Figs. 2, 3, and 5). In *B. fervidus* there was one gland duct for each of the two glands (Fig. 5). Species in which the gland surrounds the sperm pump (*M. relativa* and *A. pura*; Fig. 4) lacked a separate spermathecal gland duct, suggesting that the secretions of the spermathecal gland pass directly into the sperm pump and thence to the receptacle.

Sperm pump

In the Halictidae, the sperm pump is paired (Fig. 3) but in all other taxa examined (*Leioproctus* sp., *A. wilkella*, *M. relativa*, and *B. fervidus*) it is a single structure (Figs. 2, 4, and 5). In *H. ligatus*, a bell-shaped muscle group from one member of the pair of sperm pumps draped over the surface of the basement membrane, while fibres of the other muscle group were confined to the area surrounding the second sperm pump (Fig. 3). Serial sections showed that in this species two spherical pumps were located ventral to the spermathecal gland. Through-focus observation of the spermathecae of *A. pura* and *L. marginatum* demonstrated that paired globular sperm pumps may be typical in the subfamily Halictinae (Table 1). The sperm pump in *B. fervidus* was surrounded by flabellate muscles and wedged between paired selliform spermathecal glands (Fig. 5).

Spermathecal duct

The spermathecal duct arises from the chamber and opens into the bursa. A tributary, the spermathecal gland duct, joined the spermathecal duct in all species examined except *A. pura* and *M. relativa*. The proximal portion of the spermathecal duct traversed the thickness of the spermathecal wall,

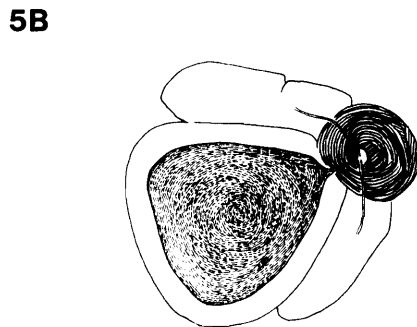
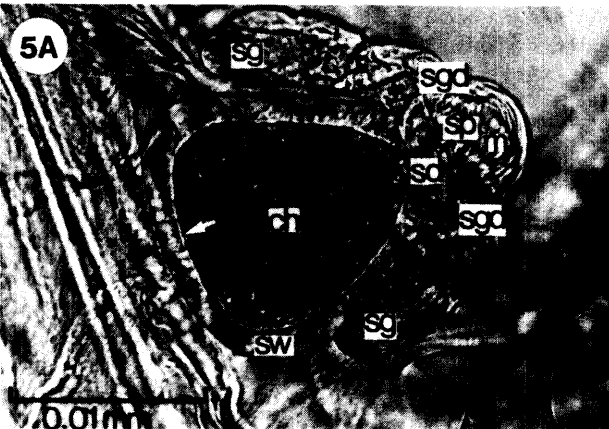
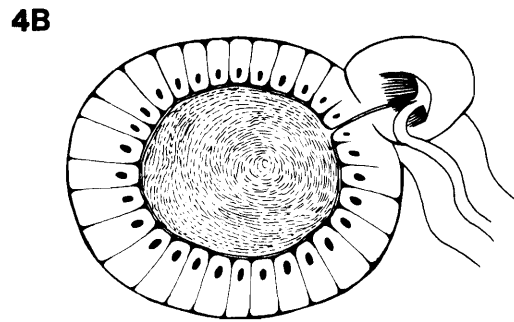
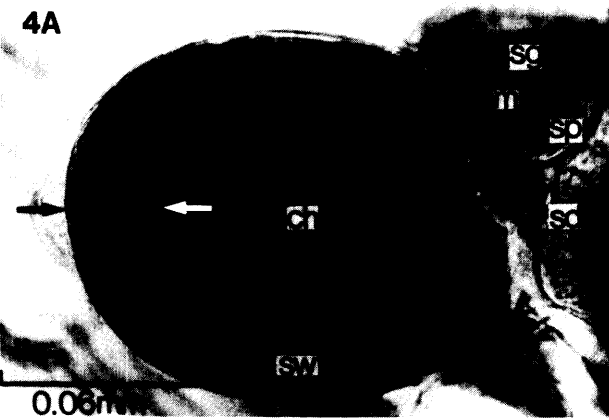
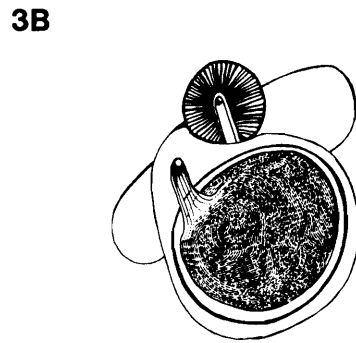
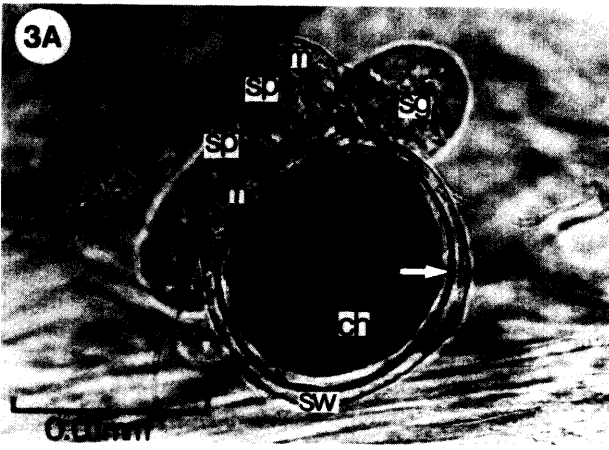
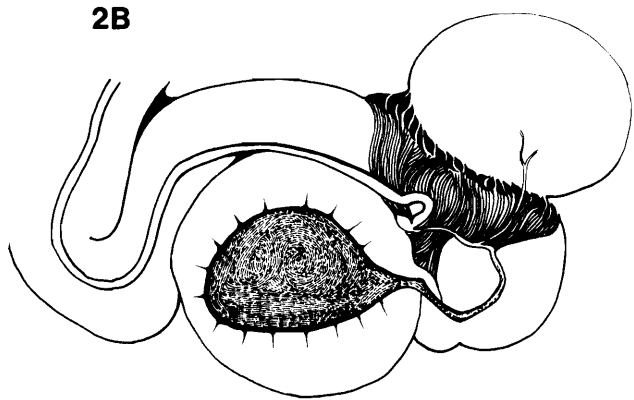
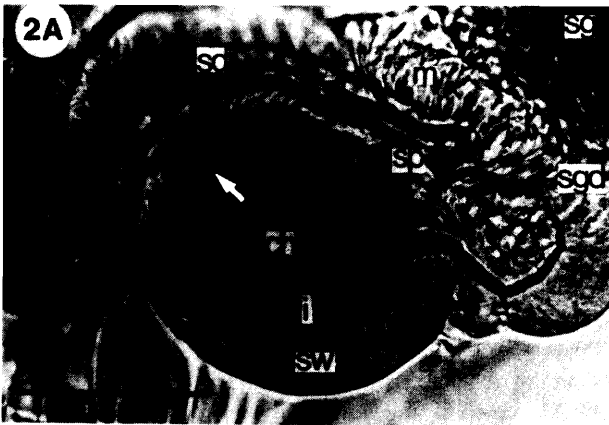
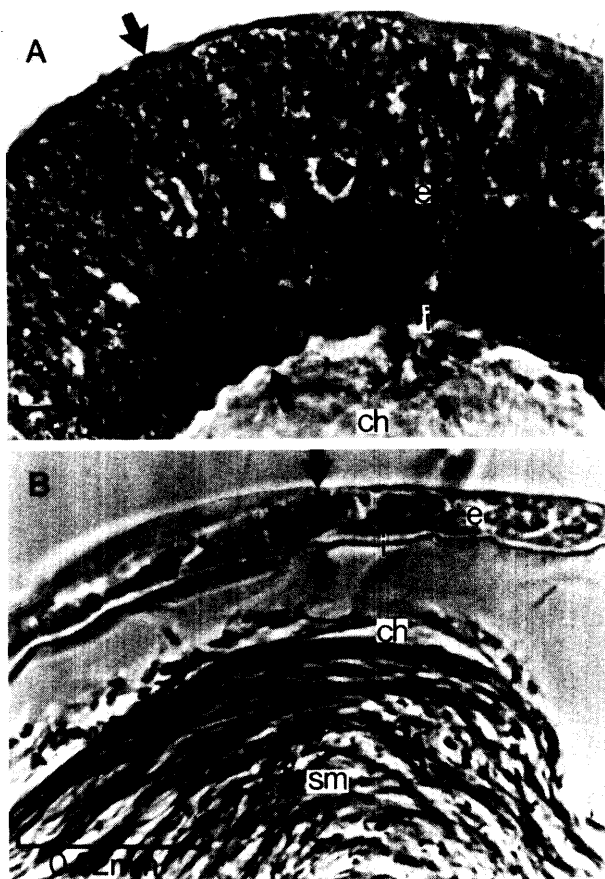


Fig. 6. Serial sections of a portion of the spermathecal receptacle of *Megachile relativa* (A) and *Halictus ligatus* (B), showing the layers of the spermathecal wall. The arrows indicate differences in spermathecal wall thickness. Mallory's one-step.



so it was longer in species with a relatively thick wall (e.g., *Leioproctus* sp., *M. relativa*, and *A. wilkella*) and shorter in species with a relatively thin wall (e.g., Halictinae). The spermathecal duct passed through the gland before proceeding to the bursa in *M. relativa* (Fig. 4). In *A. wilkella* (Fig. 2) and *Leioproctus* sp., the spermathecal duct bypassed the gland, which was separate from the receptacle. Preparations of the spermathecae of the representative halictine species showed that the spermathecal duct proceeded from the chamber and passed between the paired sperm pumps. In *B. fervidus*, the spermathecal duct arose from the chamber and passed between the paired glands before proceeding to the bursa. The spermathecal duct arising from the chamber was relatively narrower (less than $2 \mu\text{m}$) than the portion of the duct more distal to the receptacle ($\pm 4 \mu\text{m}$) in all species examined and was clearly seen in *A. wilkella* (Fig. 2).

Discussion

The production of large colonies in highly eusocial bees requires queens to have the ability to store greater numbers of spermatozoa, so we expected relatively larger spermathecal chambers in eusocial bees, and this is indeed one of our principal conclusions from this study (Table 1). The relationship

between chamber size, bee size, and the degree of sociality deserves further analysis in a wider variety of species.

Differences in the thickness of the spermathecal wall may be related to the function of the spermathecal epithelium. In general, insects have three types of spermathecal epithelium based on the location of secretory cells (Gillott 1988). In the first type, found in the American cockroach (*Periplaneta americana*), the secretory cells are distributed throughout the wall of the receptacle (Gupta and Smith 1969). In the second type, the glandular cells are restricted to a particular region of the spermathecal wall, as in the yellow fever mosquito (*Aedes aegypti*) (Clements and Potter 1967). In the third type the glandular cells form a discrete gland distinct from the receptacle, which is the condition in bees, other Hymenoptera, and many beetles, where the secretory function is specialized in the separate gland (Dallai 1975; Happ and Happ 1975). The very thin wall ($3 \mu\text{m}$) in *L. marginatum* (Halictinae), especially, suggests that the spermatheca is primarily for storage and that the secretory function has been localized in the gland. On the other hand, serial sections of the spermatheca of *M. relativa* indicate a possible secretory function of the epithelium. Maintenance of about 5×10^6 spermatozoa in the chamber of queen honey bees may require secretions of the epithelium of both the receptacle and the spermathecal gland. The finding of secretory activity in the receptacle cells of queen honey bees after mating is consistent with this, although secretory activity in these cells may cease in old bees (Dallai 1975).

While the columnar epithelium reported here might suggest a secretory function, electron micrographs of the hilar section of the spermathecal epithelium of the ant *Crematogaster opuntiae* (Wheeler and Krutzsch 1994) reveal structural details characteristic of a transport epithelium that maintains an appropriate milieu for the spermatozoa. Although the hilar region constitutes a small portion of the entire epithelium of the ant spermatheca, it is still possible that the thicker epithelium of the spermathecae mentioned here indicates a transport function as described by Wheeler and Krutzsch (1994). We interpret the thicker epithelium in the spermathecae of the megachilid and andrenid bees as evidence of a secretory epithelium because that is the most common function of the spermathecal epithelium (Gillott 1988). The sections cut through the spermathecal epithelium for light microscopy do not permit us to distinguish between the two possibilities, transport and secretion.

The spermathecal glands are long in bumble bees and longer still in *Apis* spp. If the spermathecal glands of solitary Apinae, the orchid bees (tribe Euglossini), are also elongated, this may be a significant taxonomic characteristic of Apinae. Conversely, spheroid spermathecal glands in orchid bees would indicate confinement of elongate glands to eusocial species, in which they are associated with maintenance of more sperm. Investigation of the spermathecal gland in other Apinae is needed to test these ideas.

The spermathecal muscle appears to play an important role in conveying sperm to (Rodriguez 1994) and from the spermatheca (Villavaso 1975). Camacho (1989) stated that movements of the muscles which cover the spermatheca of the Mediterranean fruit fly, *Ceratitis capitata*, may cause pressure change inside the receptacle, which would move

sperm into and out of the spermatheca. In *Megachile rotundata*, contraction of muscle fibres inserted on one side of the sperm pump may expel sperm from the chamber (Gerber and Klostermeyer 1970). Insertion of the muscles on one side of the sperm pump in *M. relativa* suggests a similar function. Annular and longitudinal muscle fibres outside the basement membrane of the spermathecal epithelium in honey bees compress the organ (Dallai 1975). A campanulate muscle group over the basement membrane in *H. ligatus* also probably compresses the spermathecal receptacle. It is assumed that contraction of the muscle fibres along the proximal tract of the spermathecal duct in *Leioproctus* sp. and *A. wilkella* act with the sperm pump in drawing sperm out of the chamber. The precise mechanism whereby this is accomplished is unclear.

Two types of spermathecal gland ducts are found in Hymenoptera (Flanders 1936; King and Ratcliffe 1969). In the first type, the gland opens directly into the spermathecal chamber, and this design is shared by all the bees in the study except *Leioproctus* sp. and *A. wilkella*. The latter two species have glands of the second type in which the duct opens half-way along the proximal tract of the spermathecal duct, the type of gland duct characteristic of parasitoid wasps (King and Ratcliffe 1969). Establishing the social evolutionary or systematic significance of this spermathecal feature depends upon further investigation of the spermathecae of other species of bees and aculeate wasps. The paired spermathecal gland ducts of both primitively and highly eusocial bees suggest greater secretory output, which may be necessary to maintain large numbers of stored sperm in eusocial species.

In summary, enlargement of the spermathecal chamber may be a socially relevant attribute in bees. Elaboration of features such as epithelial cell height, gland shape, and number of sperm pumps in bees exhibiting different levels of social behaviour may be correlated with the storage and maintenance of larger numbers of sperm as well as their more efficient withdrawal in eusocial than in non-eusocial species. Aspects of spermathecal morphology that may provide useful characters for systematic analysis include the relative thickness of the spermathecal wall, the path taken by the spermathecal duct, the number and location of the spermathecal gland(s), and the pattern of the associated musculature. Preliminary observations suggest that two sperm pumps might be taxonomically restricted to Halictinae; data on the spermathecal features of other subfamilies of Halictidae (e.g., species of Dufoureae and Nomiinae) are needed. Investigation of the spermathecae of eusocial Meliponini and solitary Euglossini (Apinae) and related long-tongued bees may reveal the origins of a thicker spermathecal wall and paired elongate glands currently known for *B. fervidus* and *A. mellifera* and provide evidence of the importance of these features for social evolution in these bees.

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