

Queen-worker differences in spermatheca reservoir of phylogenetically basal ants

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Abstract Ant queens mate when young and store sperm in their spermatheca to fertilize eggs for several years until their death. In contrast, workers in most species never mate. We have compared the histological organization of spermathecae in 25 poneromorph species exhibiting various degrees of queen-worker dimorphism. The spermathecae of both castes in all species are similar in having a reservoir connected by a sperm duct to the ovary, and a paired gland opening into this duct. The reservoir of queens typically has a columnar epithelium in the hilar region (near the opening of the sperm duct), whereas the epithelium in the distal region is cuboidal. Abundant mitochondria together with apical microvilli and basal invaginations indicate an osmoregulatory function. In contrast, the reservoir epithelium of workers is flattened throughout and lacks these transport characteristics. This single difference shows the importance of a columnar epithelium in the reservoir for sperm storage. However, our data have not revealed inter-specific variations in the

development of the hilar region linked with higher fecundity. We have found no consistent differences in associated structures, such as the spermatheca gland or sperm ducts, or in the musculature between queens and workers.

Keywords Sperm storage · Dimorphism · Mating · Haplodiploidy · Hymenoptera · Ants

Introduction

Sperm storage by female insects is an important feature of reproductive biology, as it temporally separates mating from egg fertilization. Sperm storage potentially increases fecundity as more eggs can mature over time and be fertilized with sperm from a previous mating. Although short-term sperm storage is common in animals (Birkhead and Møller 1993), only some groups have specialized storage organs. In solitary insects, females store sperm for a few days to a few months (Choe and Crespi 1997; Neubaum and Wolfner 1999), whereas social Hymenoptera are remarkable in that queens typically use stored sperm for several years (Crane 1990; Keller and Genoud 1997). Storage organs are structurally adapted to enhance sperm survival by providing shelter, a chemically stable environment and nourishment (Neubaum and Wolfner 1999).

Ant queens are sexually receptive for a short period when they are young and thus they have only a brief time-window to obtain the required amount of sperm. If a queen's sperm supply depletes prematurely, the colony will decline quickly as workers are no longer replaced. Indeed, ant queens stock enough sperm to last their lifetime, being extremely efficient in sperm use (Tschinkel 1987a,b; Tschinkel and Porter 1988). This efficiency is especially important since queens in some species live up to 20 years

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(Keller 1998). In *Crematogaster opuntiae*, a “higher” ant with long-lived and highly fecund queens, the epithelium of the spermatheca reservoir is highly columnar near the opening of the sperm duct (Wheeler and Krutzsch 1994). The ultrastructure of these columnar cells suggests active transport to regulate the chemistry of the reservoir environment, as is also found in honey bees (Dallai 1975; Wheeler and Krutzsch 1994). Sperm is stored in an inactivated state and its release seems to be linked to subsequent reactivation with products from the spermathecal gland (Koeniger 1970; Wheeler and Krutzsch 1994).

Other than part of a study on the genital tract in *Diacamma* (Allard et al. 2005), the spermatheca has not been investigated in poneromorph ants. Although queen-worker dimorphism is generally limited compared to “higher” ants, the phylogenetically basal (Bolton 2003) poneromorphs show variations in the extent of queen-worker differences (e.g. number of ovarioles; Peeters 1993). Apart from variations in fecundity, ants have dispersing and non-dispersing queens, associated with different strategies of colony foundation (Peeters and Ito 2001). Alate queens (AQ) can fly out to mate and found new colonies independently, whereas wingless “ergatoid” queens (EQ) and “gamergates” (i.e. mated reproductive workers, G) mate near their natal nest and need the help of workers to start new colonies. Ito and Ohkawara (1994) have found a relationship between the diameter of the spermatheca reservoir and the social structure in poneromorph ants. In at least bees, both spermatheca size and the development of the reservoir epithelium vary with the degree of sociality of each species (Pabalan et al. 1996; Martins and Serrão 2002).

Our goal has been to investigate, at the histological and ultrastructural level, the specialization of spermathecal structures in phylogenetically basal ants. Although poneromorph workers cannot mate in the majority of species, they retain a visually distinct spermatheca unlike the workers of “higher” ants. This makes it possible to identify spermathecal components essential for sperm storage. Queens and sterile workers have been compared. We have selected 25 species exhibiting various degrees of caste specialization in terms of fecundity (queen-worker ovarian dimorphism, colony size) and of parameters independent of fecundity (e.g. presence of wings). We expected differences in the specialization of the spermatheca associated with fecundity. Our data is compared with that for highly fecund queens of the species *Eciton* and *Crematogaster* (Hagan 1954a; Wheeler and Krutzsch 1994) and of the honey bee (Dallai 1975).

Materials and methods

Spermatheca reservoirs and glands were dissected from queens and workers of 25 species belonging to three

poneromorph subfamilies (sensu Bolton 2003; Table 1). Where possible, species were chosen to reflect intrageneric variation in fecundity. Fecundity was estimated on the basis of a queen’s ovariole number and colony size (see Table 1). In some species queens and workers differ in the number of ovarioles, and workers do not mate although they retain an identifiable spermatheca. In a few other species both queens and workers reproduce sexually (see Table 1). To elucidate which structural components of the spermatheca are essential for sperm storage, we compared ultrastructure across castes and species.

Spermathecal tissues were fixed in 2% glutaraldehyde in sodium cacodylate buffer. After postfixation in 2% osmium tetroxide and dehydration in a graded acetone series, tissues were embedded in Araldite. Semithin sections for light microscopy were stained with methylene blue and thionin. For all specimens, we investigated the histology of the sperm duct, the spermathecal gland duct, the spermathecal gland, the spermatheca reservoir and any musculature or other tissues associated with these structures. Histological sections were examined with a Zeiss Axioskop connected with a JVC camera; we used a PC running Sigma Scan Pro 5.0. We measured the thickness of the epithelium of the spermatheca reservoir near the entrance of the sperm duct (hilar epithelium) and at the opposite side (distal epithelium) and also the diameter of the spermathecal gland. Additionally, we measured head width (a standard measure for body size) on a Leica MZ-50 dissecting microscope. The ant species in our sample showed a large variation in body size that was reflected in the dimensions of their organs and even in the cell height of the epithelium in the spermatheca reservoir. We aimed to cancel out this size effect by dividing our width measurements with the head width for each species. For ultrastructural investigations, thin sections for electron microscopy were double-stained with lead citrate and uranyl acetate and examined with a Zeiss EM 900 microscope.

Results

General morphology

The spermatheca consists of a spherical reservoir, a paired spermatheca gland and a sperm duct that connects the reservoir with the common oviduct (Fig. 1a). The sperm duct functions both as the entry point for sperm during mating and for sperm release when eggs are fertilized. The spermathecal gland releases its secretion into the sperm duct, near to its opening into the reservoir. A concentration of muscle fibres is found in this region, whereas fat tissue surrounds the area where the sperm duct and reservoir connect and covers part of the spermatheca gland. Strands

Table 1 Reproductive characteristics of the investigated species, with indication of ovarian dimorphism between queens and workers (*n* number of queens sectioned, *AQ* species with alate queens, *EQ* species with ergatoid queens, *AQ+G* species with alate queens and gamergates). *AQ* and *EQ* species have sterile workers only. Gamergates are mated egg-laying workers

Subfamily	Species	Origin	Morphology of reproductives	<i>n</i>	Queen ovarioles	Worker ovarioles	Maximum colony size	Reference	
Ponerinae	<i>Anochetus</i> sp.	Indonesia	EQ	1	6	2	30	F. Ito, unpublished (<i>n</i> =2 colonies)	
	<i>Harpegnathos saltator</i>	India	AQ+G	4	8	8	65	Peeters et al. 2000	
	<i>Leptogenys kraepelini</i>	Indonesia	EQ	4	6	6	28	Ito 1997; Peeters and Ito 2001	
	<i>Leptogenys</i> sp. 12	Indonesia	EQ	2	14	14	782	Ito 1997	
	<i>Leptogenys parvula</i>	Indonesia	EQ	1	6	2–4	22	Peeters and Ito 2001	
	<i>Myopias emeryi</i>	Malaysia	AQ	1	6	0	70	F. Ito, unpublished (<i>n</i> =22)	
	<i>Myopias maligna</i>	Indonesia	AQ	2	6	6	120	F. Ito, unpublished (<i>n</i> =9)	
	<i>Myopias</i> sp. 8	Indonesia	EQ	4	6	6	19	F. Ito, unpublished (<i>n</i> =3)	
	<i>Odontomachus simillimus</i>	Indonesia	AQ	3	16	14	120	F. Ito, unpublished (<i>n</i> =4)	
	<i>Odontomachus rixosus</i>	Indonesia	AQ	6	6	6	142	Ito et al. 1996	
	<i>Odontomachus</i> sp. 4	Indonesia	EQ	1	6	6	55	F. Ito, unpublished (<i>n</i> =6)	
	<i>Pachycondyla apicalis</i>	Malaysia	AQ	3	6	6	50	Oliveira and Hölldobler 1990	
	<i>Pachycondyla astuta</i>	Mexico	AQ	1	8–11	6–10	38	F. Ito, unpublished (<i>n</i> =14)	
	<i>Pachycondyla obscuricornis</i>	Indonesia	AQ	1	6	6	130	B. Gobin, unpublished (<i>n</i> =27)	
	<i>Pachycondyla (Bothro.)</i> sp. 1	Costa Rica	AQ	4	6	6	22	Ito 1993	
	<i>Pachycondyla (Bothro.)</i> sp. 10	Indonesia	AQ+G	2	6	6	21	F. Ito, unpublished (<i>n</i> =4)	
	<i>Pachycondyla (Meso.)</i> sp. 7	Malaysia	AQ+G	1	6	6	20	F. Ito, unpublished (<i>n</i> =1)	
	<i>Pachycondyla tarsata</i>	Indonesia	AQ	3	6	6	925	Villet et al. 1989;	
	Proceratiinae	<i>Platythyrea</i> sp. 1 (near <i>parallela</i>)	South Africa	AQ	3	28	14		Braun et al. 1994
		<i>Probolomyrmex dammermani</i>	Indonesia	AQ+G	2	6	6	26	F. Ito, unpublished (<i>n</i> =13)
<i>Proceratium itoi</i>		Indonesia	AQ	1	2	2	25	Ito 1998	
Ectatomminae	<i>Gnamptogenys bicolor</i>	Japan	AQ	1	6	6	450	Peeters 1993	
	<i>Gnamptogenys costata</i>	Indonesia	EQ	1	9–14	5–12	480	F. Ito, unpublished (<i>n</i> =30)	
	<i>Gnamptogenys menadensis</i>	Indonesia	AQ	2	4	2	120	F. Ito, unpublished (<i>n</i> =5)	
	<i>Gnamptogenys moelleri</i>	Indonesia	AQ+G	5	10	6	138	Gobin et al. 1998a	
		Brazil	AQ+G	3	10	6	200	Cogni and Oliveira 2004;	
							B. Gobin, unpublished (<i>n</i> =1)		

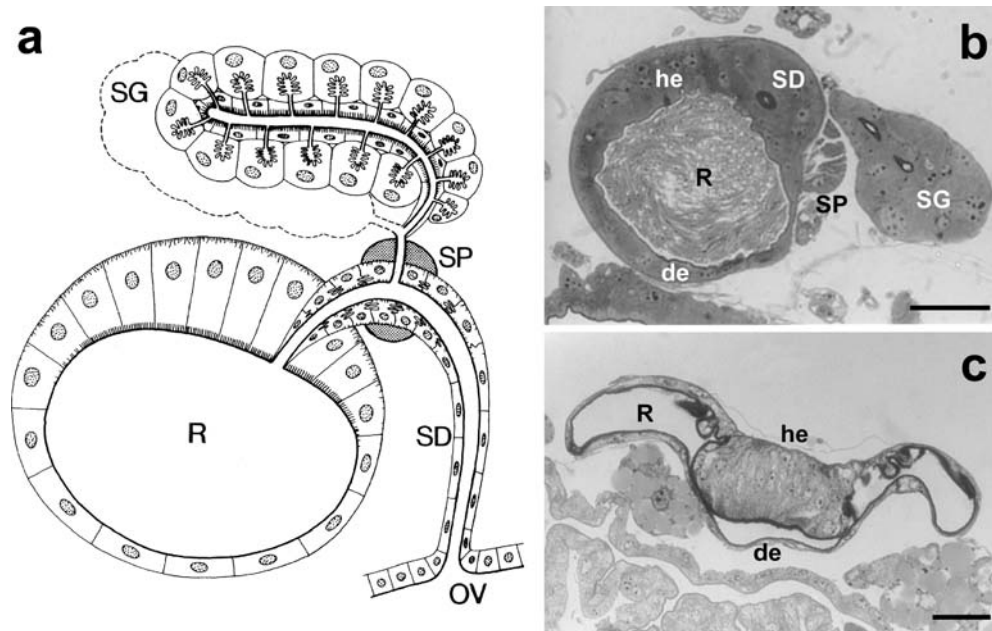


Fig. 1 **a** General organization of the spermatheca in poneromorph ants (*R* reservoir, *SD* sperm duct, *SG* spermatheca gland, *SP* sperm pump, *OV* oviduct). **b** Semithin section through spermatheca reservoir of a queen of the species *Leptogenys parvula* (*R* reservoir, *SD* sperm duct,

SG spermatheca gland, *SP* sperm pump, *he* hilar epithelium, *de* distal epithelium). *Bar* 20 μm . **c** Semithin section through spermatheca reservoir of a queen of the species *Gnampptogenys bicolor* (*R* reservoir, *he* hilar epithelium, *de* distal epithelium). *Bar* 20 μm

of muscle fibres surround the reservoir. Surprisingly, the spermatheca in workers has an identical general morphology to that of queens, including the appearance of ducts, gland and muscles. However, it differs in the cellular organization of the epithelium of the spermatheca reservoir (cf. Fig. 3d,e).

Spermatheca reservoir

The spermatheca reservoir of all investigated queens (AQ and EQ) shows a distinctly columnar hilar epithelium in the region near the opening of the sperm duct, whereas the distal region of the reservoir consists of flattened cells (Fig. 1a). The transition between columnar to flattened epithelium in queens is generally gradual (Fig. 1b; see also Fig. 3a), except in all *Gnampptogenys* species studied, in which the transition is discrete (Fig. 1c). The relative thickness of the hilar and distal epithelium varies across species. However, this variation cannot be attributed to fecundity (number of ovarioles; Fig. 2, Table 1). Across species, both AQ and EQ have a similar range of hilar epithelium specialization. At a generic level, differentiation between hilar and distal epithelia in queens of the highly dimorphic *Pachycondyla tarsata* is similar to that in *Pachycondyla* species with low dimorphism (Table 1, Fig. 2). A similar lack of differentiation exists between three species of the genus *Odontomachus* having AQ or EQ; the lack of ovarian dimorphism in *O. rixosus* is not associated with lower spermatheca

specialization. This similarity in reservoir structure is also found in three species of *Leptogenys* with different reproductive characteristics.

The ultrastructural organization of the epithelial lining of the spermatheca reservoir is similar in all species investigated. The columnar cells of the hilar region are characterized by an abundance of mitochondria in their apical cytoplasm (Fig. 3b). Smooth endoplasmic reticulum and Golgi apparatus are weakly developed. The nuclei occur in the basal cell region and are generally rounded, with occasional obvious nucleoli (Fig. 3b). Large glycogen pockets are found in the basal region of all species. The apical cell membrane displays a microvillar differentiation, whereas the basal plasmalemma shows conspicuous invaginations (Fig. 3b). The distal epithelium, in contrast, clearly lacks apical microvilli and basal invaginations, and only a few mitochondria are found (Fig. 3c). In both the hilar and distal epithelia, the cell membrane of adjoining epithelial cells strongly intertwines near their apical side. The cuticular lining of the entire reservoir shows no variation in thickness, whether in the hilar or in the distal regions.

In contrast, the reservoir epithelium of workers that never mate lacks a columnar region altogether (Fig. 3d, cf. Fig. 3b,e). The entire reservoir wall has flattened cells containing only a few organelles and appears similar to the distal epithelium in queens. The lack of subcellular structures shows that these cells lack significant activity. Thus, the type of cells (flattened as in Fig. 3d or columnar

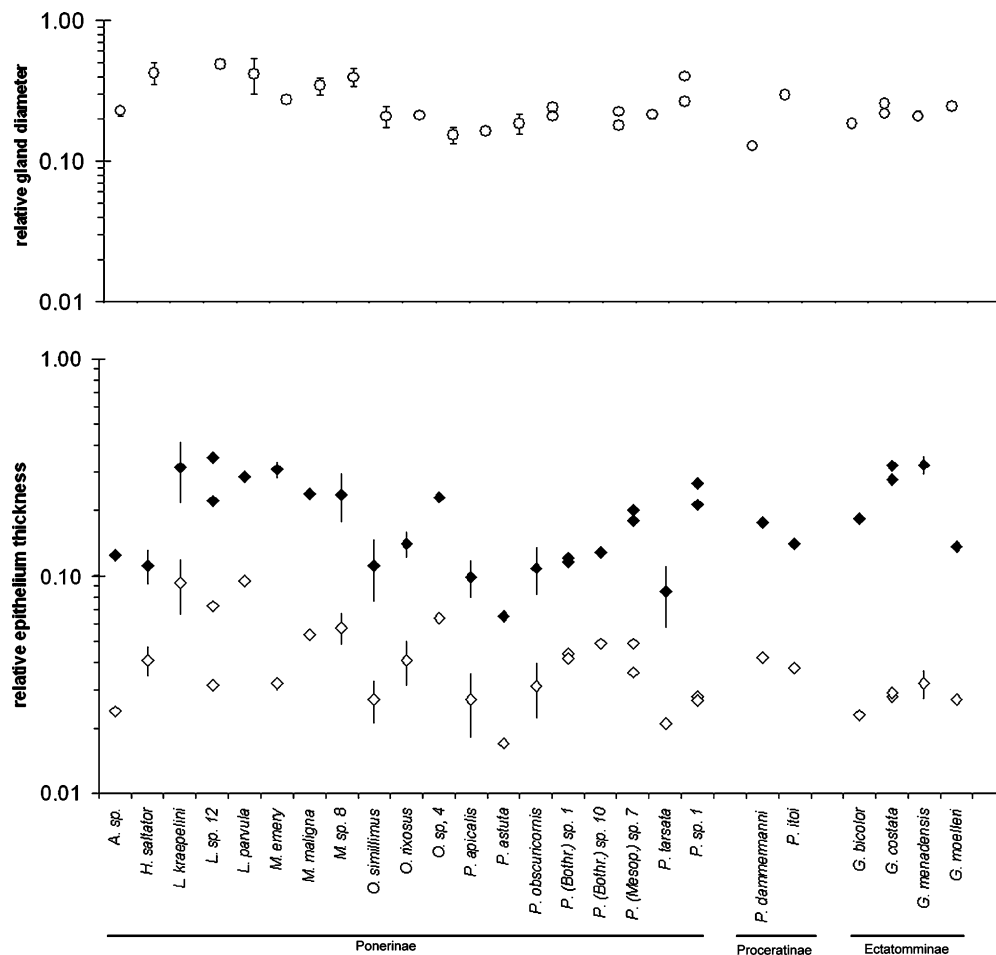


Fig. 2 Log plots of the relative hilar (*black diamonds*) and distal (*open diamonds*) epithelium thickness and relative gland diameters (*open circles*) of spermathecae of 25 poneromorph ant species (see

Table 1 for details). Values represent the ratio of epithelium thickness over head width (*bars* standard deviations). Separate values are shown when only two individuals were sampled

as in Fig. 3a,b and e) in the reservoir epithelium is linked to the functionality of the spermatheca reservoir.

Spermatheca gland

The two elongate branches of the spermatheca gland each have a central duct, with both ducts merging just before opening into the sperm duct. The diameter of the glandular branches, relative to body size, is variable among species. Again, no pattern related to differences in fecundity or queen type can be observed (Fig. 2), whereas an identical structural and ultrastructural organization is found in workers.

The gland is formed by type-3 secretory units (classification of Noirot and Quennedey 1974). This common type of bicellular organization in insect glands features the release of secretory products from individual gland cells through the ductule formed by an accompanying duct cell. These ductules in turn open into the central glandular duct, which is lined with cuticle and a single layer of flat epithelial cells (Fig. 1a). Fat tissue occurs in close association with the

spermatheca gland, whereas nerves and tracheoles surround the gland cells and penetrate between them.

The glandular cells have large polygonal nuclei and are especially characterized by the highly conspicuous end apparatus, which is formed by a central cuticular ductule surrounded by microvilli (Fig. 4a,b). This structure, which is characteristic for type-3 exocrine glands, forms the connection between the glandular cell and the duct cell. The microvilli of the end apparatus often form long extensions into the cytoplasm (Fig. 4b) and are either tightly arranged or distorted with electron-lucid material, even within the same individual. Rounded to elongate mitochondria are mainly concentrated around the microvilli of the end apparatus but are scarce elsewhere in the cytoplasm. Few organelles occur, with some scattered ergatoplasmic strands and some free ribosomes (Fig. 4b). Glycogen pockets are scattered thinly throughout the cytoplasm. Secretory material is restricted to a few small mostly electron-lucid droplets, whereas a few lamellar vesicles can occasionally be seen. The cuboidal epithelial

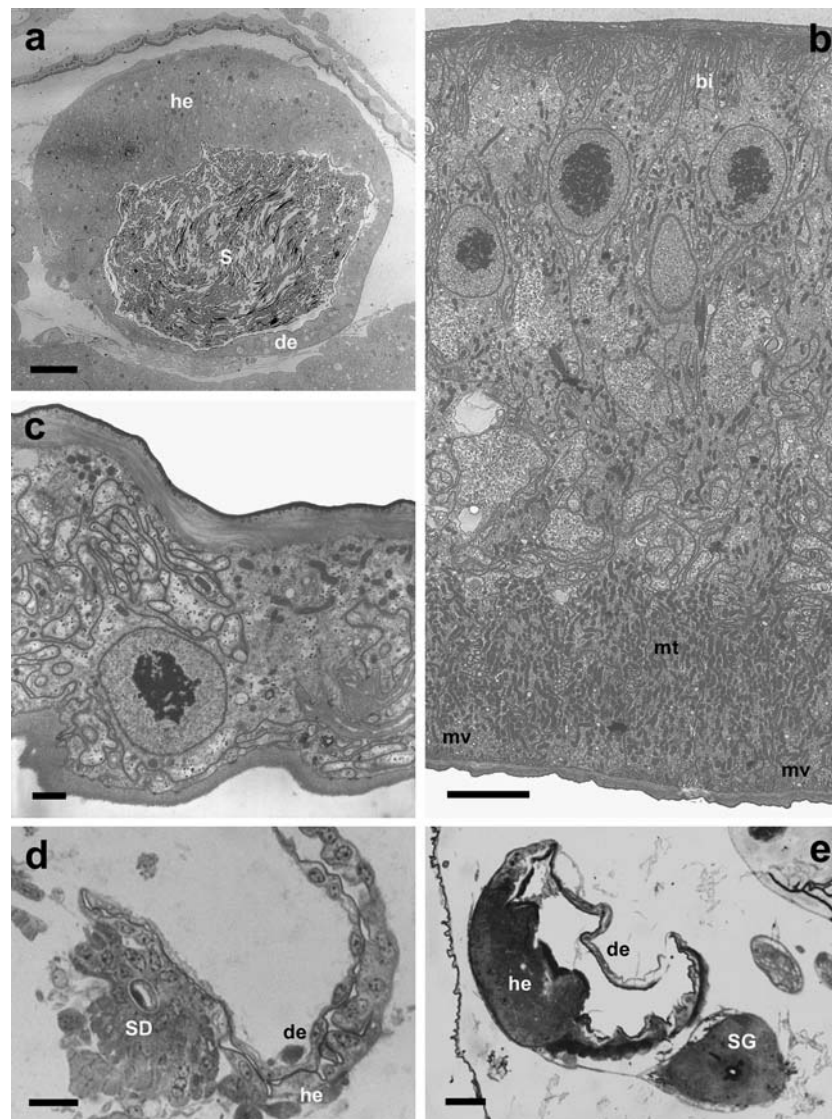


Fig. 3 Electron micrographs of the spermatheca reservoir wall. **a** Overview of the spermatheca of a queen of the species *Leptogenys parvula* with hilar (*he*) and distal (*de*) epithelia (*S* sperm). Bar 10 μ m. **b** Columnar cells of the hilar epithelium in a queen of the species *Pachycondyla tarsata*. Note the abundant mitochondria (*mt*), microvilli (*mv*) and basal invaginations (*bi*). Bar 5 μ m. **c** Cuboidal cells of

the distal epithelium of a *P. tarsata* queen, lacking most organelles. Bar 1 μ m. **d** Overview of the spermatheca reservoir of a *P. tarsata* worker, with a reduced epithelium lacking differentiation between hilar (*he*) and distal (*de*) regions (*SD* sperm duct). Bar 10 μ m. **e** Overview of a queen of *Myopias* sp. 8 with a columnar hilar (*he*) and a flattened distal (*de*) epithelium (*SG* spermatheca gland). Bar 10 μ m

cells lining the central duct of the spermatheca gland contain many round to oval mitochondria and dense apical microvilli lined with cuticle. The epithelial cells are tightly connected by interdigitating intercellular junctions in the apical region (Fig. 4d).

Sperm duct and musculature

The sperm duct connects the sperm reservoir to the oviduct. Poneromorph ants lack a flap-like structure at the boundary between the reservoir and duct. The central duct of the spermatheca gland opens into the sperm duct near its

opening into the sperm reservoir. Strong muscles of the sperm pump surround the sperm duct at this point (Fig. 4e, MF2), whereas some muscle fibres attach directly onto the duct (Fig. 4e, MF1). Ultrastructural observation of this region shows bundles of parallel microtubules within the epithelial cells in those regions in which muscle MF2 attaches to the epithelium (Fig. 4f). The thick cuticle lining the lumen shows numerous taenidia-like ridges in this region, presumably giving added strength to keep the duct wall open. This is especially clear in *Odontomachus* (Fig. 4e). Further along the sperm duct, outside the region of the sperm pump, the cuticle smoothens once again. The

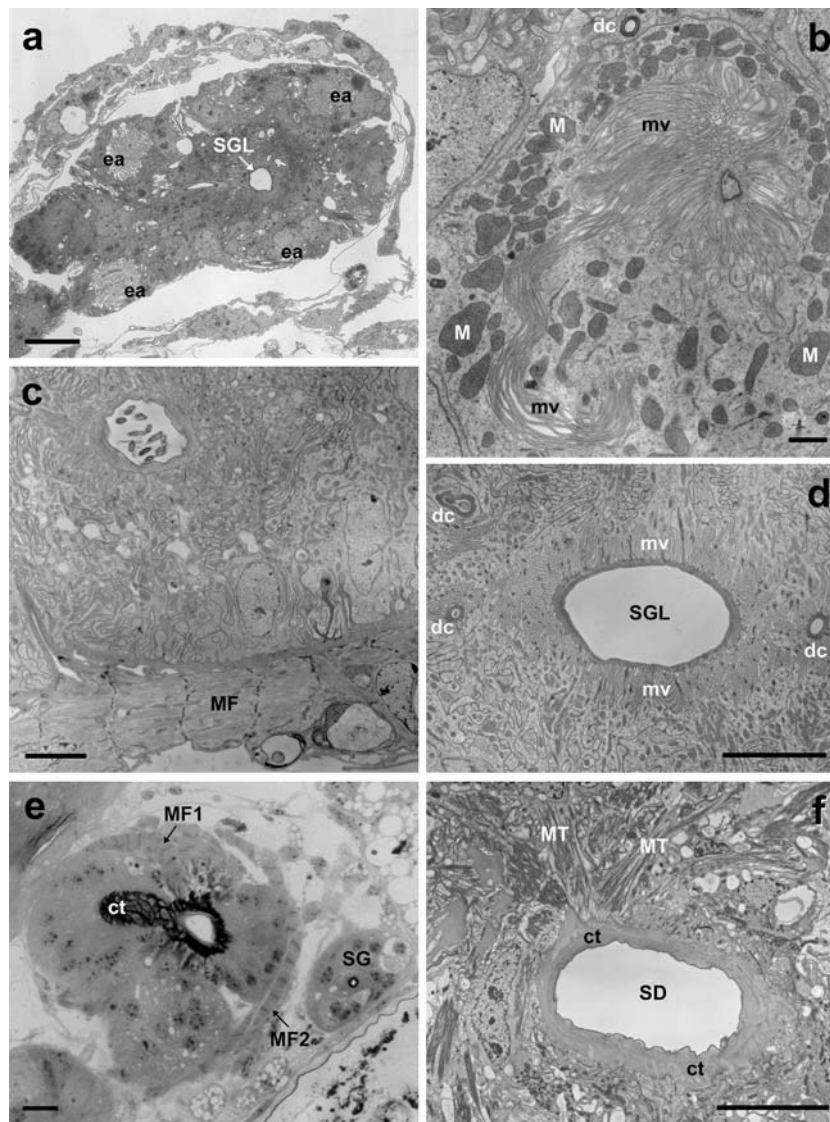


Fig. 4 Micrographs of spermatheca gland (SG) and sperm duct (SD). **a** Spermatheca gland of *Harpegnathos saltator* queen (ea end apparatus, SGL spermatheca gland lumen). Bar 10 μ m. **b** End apparatus with long microvilli (mv) and surrounding mitochondria (M) in an *Odontomachus rixosus* queen (dc duct cell). Bar 1 μ m. **c** Sperm duct with surrounding muscle fibres (MF) in ergatoid queen of *Myopias* sp 8. Note sperm in lumen (top left). Bar 5 μ m. **d** Cross section through spermatheca gland duct with epithelial cells displaying apical microvillar (mv) border in queen of *Pachycondyla* (=Bothropo-

nera) sp. 1 (dc duct cells). Bar 5 μ m. **e** Semithin section through sperm duct of *O. rixosus* queen. Region of the sperm pump with muscle fibres directly attaching to the duct (MF1) and indirectly encompassing the entire duct (MF2). Note the irregular differentiation of the left part of the cuticle (ct). Bar 10 μ m. **f** Electron micrograph of the sperm pump region of *Pachycondyla apicalis* worker. Parallel bundles of microtubules (MT) in the epithelial cells transmit the muscular pulling forces onto the cuticle (ct). Bar 5 μ m

epithelial cells of the sperm duct show some basal invaginations and apical microvilli. The cells contain a round nucleus, many mitochondria but few organelles or inclusions (Fig. 4c).

Discussion

The overall structure of the spermatheca is comparable in the queens of all 25 investigated poneromorph ant species.

The spermatheca reservoir wall contains a hilar region, with highly columnar cells, like that described in the myrmicine ant *Crematogaster opuntiae* (Wheeler and Krutzsch 1994). Basal invaginations and apical microvilli enlarge the contact surface with the haemolymph and lumen, respectively, whereas the presence of a large number of mitochondria suggests important cellular activity that requires energy. These are characteristic features of transport epithelia (Berridge and Oschman 1972), as described in *Crematogaster* and bees (Dallai 1975; Wheeler and

Krutzsch 1994; Schoeters and Billen 2000). The large glycogen stores in the columnar cells might provide the energy needed for this transport. The close association of numerous mitochondria with the apical cell membrane indicates active pinocytotic transport of material between the cells and the spermatheca lumen. The apparent lack of organelles such as endoplasmic reticulum and Golgi apparatus suggests little modification of the transported material. These features further indicate an osmoregulatory function, which is possibly essential in providing a suitable environment for prolonged sperm survival. In contrast, the flattened epithelial cells of the distal region lack microvilli and invaginations and contain few mitochondria. Their role is presumably limited to providing a physical barrier. The organization of the reservoir epithelium is different from that found in bees (Dallai 1975; Pabalan et al. 1996; Martins and Serrão 2002). All bees exhibit a reservoir entirely lined with columnar epithelium and the thickness of this epithelium correlates positively with colony size in a given species. Army ant queens are the most specialized egg-layers among the Hymenoptera, with *Eciton* queens having up to 2400 ovarioles (Hagan 1954a) that can produce colonies of 10^6 workers (Schneirla 1971). Early histological studies of this species (Hagan 1954b; Whelden 1963) suggest that at least part of the spermatheca reservoir epithelium is columnar but no clear information is given with regard to its extent.

In poneromorph queens and sterile workers, the paired spermatheca gland is composed of clusters of bicellular units, in which each secretory cell releases its products via an end apparatus into the duct of its accompanying duct cell. The microvilli of the end apparatus are extremely long in comparison to those in many other exocrine glands in social insects (see Billen and Morgan 1998). The cytoplasm, however, is remarkably poor in organelles. As in *C. opuntiae*, the presence of glycogen pockets suggests high energy requirements for secretion or may be part of the secretory product itself (Wheeler and Krutzsch 1994). Numerous fat cells and tracheoles closely associated with the gland cells give an indication of secretory activity. Fat stores in cells surrounding the spermatheca are depleted after mating in the ant *Gnamptogenys menadensis* (Gobin et al. 1998b). The epithelium lining the central duct of both gland arms has well-developed apical microvilli and numerous mitochondria, suggesting that the secretion in the lumen is further modified. Both gland ducts merge just before opening into the sperm duct. The close association between the openings of both reservoir and gland into the sperm duct suggest that sperm release and gland secretion are functionally linked. The secretion of the spermathecal gland may serve to reactivate sperm passing through the sperm duct upon release. In the honeybee, operative removal of the spermatheca gland inhibits egg

fertilization but does not affect sperm survival in the reservoir (Koeniger 1970).

In queens and in sterile workers, two muscles are associated with the sperm duct near its opening into the reservoir and thus form the sperm pump that controls the release of sperm and secretions of the spermathecal gland. Circular muscles exert their squeezing forces onto the duct to close off the spermatheca. The duct can open up either by a spring-like function of the taenidia-like reinforcements in the cuticle or by forces from muscles connecting directly to the duct. The pulling forces of this muscle (MF2) are transmitted onto the cuticle through parallel bundles of microtubules in the epithelial cells. This corresponds to the common structural feature in insects where muscular forces pull on the cuticular layer (Lai-Fook 1967). The haplodiploid social Hymenoptera are extremely efficient in the control of sperm release (Ratnieks and Keller 1998), as fertilization controls the sex of offspring: unfertilized eggs become males, whereas fertilized eggs give rise to females. In contrast, the diploid *Locusta migratoria* has no control over fertilization and sperm release is activated through a neural loop whenever an egg passes the genital chamber (Clark and Lange 2001). The sperm pump of ants is unlikely to play an active role in sperm uptake, as the filling of the spermatheca is slow. In the myrmicine ant *Acromyrmex versicolor*, it takes 1–5 h after mating for all sperm to reach the spermatheca (Reichardt and Wheeler 1996).

The epithelium of the short sperm duct in ponerine ants shows some secretory activity near the reservoir but far less than that of the columnar duct epithelium in bumblebees (Schoeters and Billen 2000). In *Bombus*, the enlarged epithelium of the sperm duct secretes polysaccharides to activate sperm. Near the oviduct, the duct epithelium becomes squamous, as reported in *C. opuntiae* and *Apis* (Dallai 1975; Wheeler and Krutzsch 1994). *Eciton* queens have extremely long sperm ducts, although of similar cellular organization (Hagan 1954b). The valvular flap marking the transition between sperm duct and reservoir in *C. opuntiae* and *E. burchelli* (Wheeler and Krutzsch 1994; Hagan 1954b) is absent in all investigated Ponerinae.

When we compare spermatheca morphology among queens of poneromorph ants, we find no structural differences. The hilar epithelium of the spermatheca in all *Gnamptogenys* species (subfamily Ectatomminae) has a more pronounced thickening compared with that of other genera. When comparing EQ and AQ among the poneromorphs, we find no variation in spermatheca specialization. The absence of wings is indeed a specialization for dispersal and should not affect sperm storage capability (Peeters and Ito 2001). Queens with higher fecundity, as measured by ovariole numbers, do not exhibit increased

development of their hilar epithelium. Even though a relationship between spermatheca size and fecundity has been found in ponerine ants (Ito and Ohkawara 1994; Ito 1997), this is not reflected in the specialization of the reservoir epithelium (see Fig. 2). We have to take care in interpreting this lack of variation, as poneromorph ants differ little with regard to ovariole numbers. Species of “higher” ants with highly specialized queens featuring a hundredfold more ovarioles must be studied in order to establish whether any such relationship exists.

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