

The palpal organ of male spiders (Arachnida, Araneae)

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Abstract

Current hypotheses concerning the function of the palpal organ are reviewed with special reference to histological and experimental studies. The results of histological and electron microscopic studies from the palpal organs of *Liphistius trang* and *Ryuthela nishihirai* are presented and discussed with respect to their functional and phylogenetic aspects. The functioning of the palpal organ is divided into two phases: erection/deflection of the bulbus, and induction/ejaculation of the sperm from the spermophore.

Key words: Araneae, Mesothelae, *Liphistius*, histology, electron microscopy

INTRODUCTION

The role of the palpal organ in sperm transmission was apparently first observed and described by Menge (1843). Later on, efforts were made to understand the underlying mechanism: Bertkau (1878) recognized the important role of the hemolymph, but he also attributed the ejaculation of sperm to it, consequently, he was not able to explain the induction of sperm into the bulbus. At that time, it was still believed, that sclerotized bulbs like that of *Segestria* had to be considered as the original (plesiomorphic) condition (Bertkau 1875), a view which became more widespread through Comstock's work and his famous book on spiders (Comstock 1912). After the histological characterization of the hematodocha (Wagner 1888), the presence and the role of muscles in the erection of the palpal bulbs of mygalomorph spiders was first recognized by Szombathy (1913, 1915). He homologized these muscles with those of the claw, recognized the absence of one muscle (m. contrator bulbi) in the palps of an entelegyne spider (*Agelena*) and discovered the existence of gland cells inside the bulb. Osterloh (1922) performed another histological study on the

palpal organ and recognized that the glands surrounding the spermophore amazingly differ before and after copulation, thus suggesting a role for gland secretion during ejaculation, a mechanism previously proposed by Szombathy (1915).

The development of the palpal organ was studied histologically by Harm (1931) in what was believed to be a primitive palpal organ (*Segestria*), and in a more advanced spider (*Evarcha*) (Harm 1934). Homann (1935) approached the functional problem from an experimental viewpoint, emphasizing the role of hemolymph pressure. Microdissection, histology and histochemistry were applied by Cooke (1966) in order to elucidate the structure and function of the *Dysdera* palpal organ. He disclosed the palpal gland and a pore system through the spermophore cuticle in *Dysdera*. In a very extensive study Lamoral (1973) described the histology of a sparassid palpal organ, explained the function of the hematodocha and carried out experiments in order to evaluate different functional hypotheses.

Although it was known for many years, how complicated the palpal organ was in liphistiids (Abraham 1923), it took more than

half a century until such organ was studied functionally. Living senile males, maceration techniques and SEM were used for this purpose (Haupt 1978, 1979). At about the same time another SEM study of a *Liphistius* palpal organ was undertaken (Kraus 1978), and eventually palpal organs were classified as: 1) hydraulic bulbs, 2) glandular bulbs or 3) intergrading stages (Kraus 1984). A schematic drawing of a hydraulic bulb is shown in Fig. 1. There has been much guesswork on the function of palpal organs which has again entered the secondary literature. For this reason we shall concentrate here only on histological, electron microscopic and experimental work.

MATERIAL AND METHODS

Liphistius trang (Platnick & Sedgwick, 1984) was collected in the south of Thailand, and males were fixed for histological purpose shortly after the final moult and prior to the induction of sperm into the spermophore. Fixation was for 2h in Bouin's solution, the embedding media was Paraplast, and staining was carried out with Heidenhain's Azan. The examination was under a Zeiss Forschungsmikroskop II. *Ryuthela nishihirai* male palpal organs after induction of sperm were fixed in 12% glutaraldehyde (Serva) in 0.1 M phosphate buffer at pH 7.4, postfixed in 2% osmiumtetroxide in corresponding buffer, and embedded in Durcupan. Ultrathin sections were examined under a Philips EM 208 electron microscope.

RESULTS

The bulbus of *Liphistius* is connected to two strong tendons belonging to the muscles M 29 (m. contractor bulbi genitalis) and M 30 (m. flexor unguium). They consist of meso- and endocuticle and are still surrounded by epidermal cells. The latter muscle (M 30) inserts on the dorso-distal side of the tibia (Fig. 2A). A large hemolymph vessel leads directly into the bulbus, i.e. the hematodocha (Fig. 2B). The lumen of the spermophore does not show any evidence for the presence of a secretion prod-

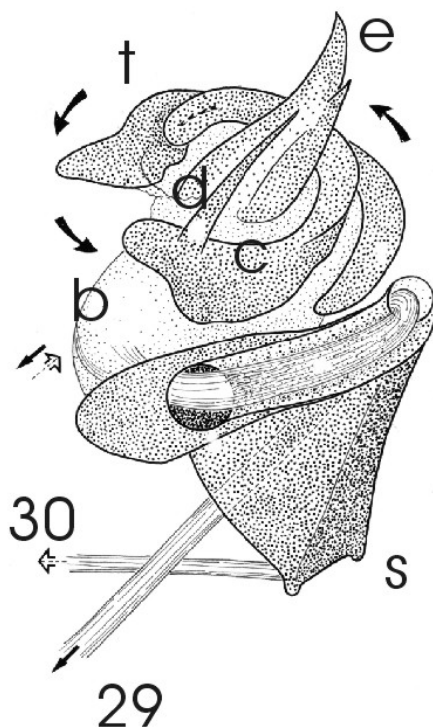


Fig. 1. Schematic drawing of the hydraulic palpal organ with black arrows indicating the erection of the bulb and light arrows the return of the bulb (from Haupt, 1979). b = bulb, c = contrategulum, d = conductor, e = embolus, s = subtegulum, t = tegulum, 29 = tendon of musculus contractor bulbi genitalis, 30 = tendon of musculus flexor unguium.

uct (Fig. 2C). Different layers can be distinguished in the cuticle of the spermophore. In its distal part facing the lumen we find red-stained mesocuticle below the epicuticle, which can be transformed locally into yellowish exocuticle. The inner (basal) layer of the spermophore cuticle, as well as the cuticle of the hematodocha, consist of blue-stained (i.e. unsclerotized) endocuticle (Fig. 2C). At the freshly moulted stage studied in *Liphistius*, cells inside the bulb are comparatively small and numerous, and there is no evidence of gland cells or any glandular function.

Electron microscopic results were obtained from a palpal organ of *Ryuthela nishihirai* after the induction of sperm. They reveal that the

lumen of the spermophore is lined by epicuticle, which is mainly made up by a dense layer. The distal part of the spermophore is made of rather dense layers of chitin fibrils, in the proximal part of the spermophore these are limited to the basal region, while the apical region consists of a very loose arrangement of chitin fibrils embedded in a clear matrix (Fig. 3). Facing the epicuticular layer the deposition of chitin fibrils is continuous. Glandular cells found inside the bulbus in *Ryuthela nishihirai* contain many glycogen platelets (Fig. 4).

DISCUSSION

In mesothelid spiders it has been shown experimentally (Haupt 1978, 1979), that muscular contraction is involved in the erection of the bulb, and another muscle is responsible for its return to the original position. Such movements are almost certainly supported by hemolymph pressure as well, a view which is supported by the presence of a large hemolymph vessel entering the hematodocha. The muscles concerned are present in Mygalomorphae (Szombathy 1913, 1915), and even in *Gradungula* (Huber 1994) both muscles are still present, thus this shows a plesiomorphic situation. M 29 (musculus flexor ungui, m. contractor bulbi genitalis) is not found any longer in *Agelena* (Szombathy 1913, 1915), but the musculus extensor bulbi (M 30) is still present. The same situation is known from two hirsutiids and one oecobiid (Huber 1994). On the other hand, in representatives of 35 entelegyne families no traces of muscles (or their tendons) were found (Huber 1994). These observations lead to the conclusion that the erection/return of the bulb, although caused by muscular contractions in the plesiomorphic condition, is eventually only carried out through hemolymph pressure, emphasizing the role of fibrous and elastic components of the hematodocha as proposed by Lamoral (1973). The important role of hydrostatic pressure has been proved experimentally much earlier (Homann 1935), although it applies more directly to the hematodocha, since a spe-

cial blood vessel leads into it (Fig. 2B).

Concerning induction and ejaculation of sperm from the spermophore a 'pipette' mechanism has been proposed for mesothelid spiders (Haupt 1979). This could readily explain induction and ejaculation, especially since the spermophore apparently does not contain secretion products prior to induction (Fig. 2C). The special structure of the spermophore cuticle in its proximal part (Fig. 3) suggests a much higher flexibility of these parts as compared to the distal region of the spermophore. Therefore, the application of pressure to the thin-walled bulbal part and its transfer to the proximal spermophore may readily cause the ejaculation of sperm (Haupt 1979). As the bulbal glandular tissue is not yet differentiated after moulting, and the electron microscopic results of later stages show mostly glycogen platelets in the glandular cells, there seems to be no evidence of glandular involvement in movements of sperm into or from the spermophore.

Compared to Mesothelae, in mygalomorph spiders the spermophore is considerably narrow and elaborate, which would make capillary mechanisms more likely. This is of course, pure speculation, as experimental studies in this group are lacking. Szombathy (1915) preferred the hypothesis of a glandular mechanism, since the spermophore of *Agelena* was found to be covered by glandular tissue, and a dark blue secretion product (Orange-G staining) was found inside the spermophore. Glandular tissue has also been reported from the palpal organ of the ctenizid *Nemesia caementaria* (Lopez & Juberthie-Jupeau 1980/81) and the haplogyne *Dysdera* (Cooke 1966). Nevertheless, glandular tissue found in the bulb of *Ryuthela nishihirai* (Fig. 4) contains large amounts of glycogen platelets, thus suggesting a nutritional role of the gland. The nutritional role of a bulbal gland in mesothelid spiders is also favored by the fact that glandular tissue is not yet differentiated after the final moult when the induction of sperm would soon be immanent.

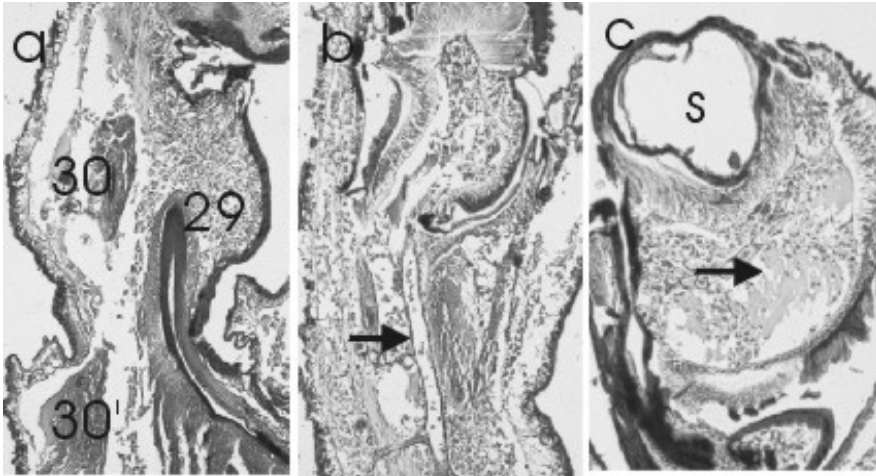


Fig. 2. (A) Tendon of M 29 (29) entering the cymbium of the palpal organ of *Liphistius trang*. 30 tendon and (30') muscle M 30. Azan staining. (B) Hemolymph vessel (arrow) in the cymbium entering the hematodocha. *Liphistius trang*. Azan staining. (C) Cross-section of spermophore (s) from palpal organ of *Liphistius trang* fixed just after moulting to adulthood. Note the small and undifferentiated cells in the bulb lumen (arrow). Azan staining.

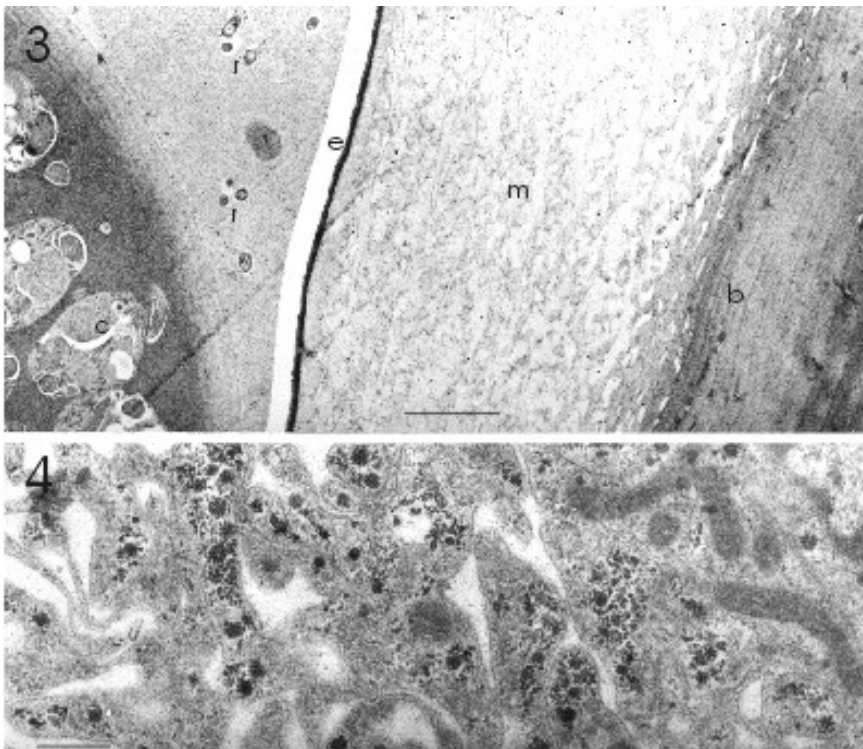


Fig. 3. Cross-section of the proximal part of the spermophore. *Ryuthela nishihirai*. b = basal part of endocuticle, c = coenospermium, e = epicuticle, m = meshy part of endocuticle, r = rickettsia-like microorganisms. Bar: 4 μ m.

Fig. 4. Glycogen platelets in gland cells found in the bulb of *Ryuthela nishihirai*. Bar: 2 μ m.

Table 1. Palpal mechanisms as revealed by histology/electron microscopy and experimental studies. Note: No experimental studies are available for Mygalomorphae.

Palpal mechanism	Taxa		
	Mesothelae (<i>Liphistius</i> , <i>Ryuthela</i>)	Mygalomorphae (<i>Theraphosidae</i> sp., <i>Nemesia</i>)	Entelegynae (<i>Palystes</i> : <i>Sparassidae</i> , various families)
Erection of bulbus	muscle M 29 and increasing hemolymph	muscle M 29 (and increasing	increasing hemolymph pressure, structure of
Return of bulbus	muscle M 30 and decreasing hemolymph pressure	muscle M 30 (and decreasing hemolymph pressure ?)	decreasing hemolymph pressure, structure of hematodocha, if present:
Induction of sperm	pipette mechanism	[capillarity ?], (resorption of secretion	Capillarity and/or resorption of secretion
Ejaculation of sperm	pipette mechanism, application of	gland secretion (induced by endocrine	gland secretion induced by endocrine factor
Author	Haupt 1978, 1979,	Szombathy 1915; Lopez	Lamoral 1973;

Concerning entelegyne spiders, mechanisms of induction and ejaculation of sperm from the spermophore were largely discussed by Lamoral (1973). During his study of the sparassid *Palystes castaneus* he distinguished two glands. While the 'syncytial' gland is situated around cuticular pores belonging to the reservoir, the vesicular gland is situated around the fundus pores and the proximal end of the reservoir. The syncytial gland is believed to provide a lubricating, nourishing, bathing fluid, while the vesicular gland secretion is thought to drive the sperm out. Suhm et al.'s (1995/96) study of *Amaurobius* also found two kinds of glands, but the bulbus gland was shown to produce the plug inserted into the female genital tract after copulation. The spermophore gland on the other hand is believed to be responsible for induction and ejaculation of sperm. It is adjacent to a region of thin cuticle.

Reports are somewhat contradictory: while Lopez (1977) described the spermophore as being filled by a secretion product prior to the induction of sperm, Lamoral (1973) readily

managed to induce sperm (in the sparassid *Palystes castaneus*) simply through capillary forces into just over 80% of the spermophore length without any glandular action, and he also succeeded in filling the whole spermophore once it was not varnished artificially. Such function would be unlikely in the presence of fluid in the spermophore lumen of entelegyne spiders. Capillarity can, at best, only be accepted for the first filling. Subsequently, the spermophore lumen must be filled with a secretion product and a resorption of this would need to be postulated. Possibly, the repeated fillings of the spermophore in several theridiid spiders (Knoflach pers. comm.) can only be explained that way.

Pulsation of the hematodocha during the ejaculation has been observed by many authors. For this reason a particular and temporary increase in hemolymph pressure must be postulated. On the other hand, in entelegyne spiders it was not possible to induce ejaculation experimentally simply by increasing the hemolymph pressure inside the bulbus. Therefore, the action of gland secretion has been

postulated (Lamoral 1973). Secretion of the vesicular glands is probably caused by a hormone, as can be shown by injecting hemolymph from one copulating male into another (Cooke 1966; Lamoral 1973). Osterloh (1922) observed striking differences in gland morphology when he compared males before and immediately after copulation.

These contradictory observations may show that the mechanism of sperm ejaculation in entelegyne spiders may be far from clear. Possibly at least two different mechanisms are involved. In an approach to elucidate the palpal function it may be useful not only to distinguish clearly between different suborders and even families, but anatomy and histology of the palpal organ also clearly necessitate the distinction of two mechanisms which are basically independent from each other (Table 1):

- 1) erection of the bulbus from the alveolar region of the cymbium and its return into it; and
- 2) the induction/ejaculation of sperm from the spermophore inside the bulbus.

Moreover, these mechanisms were apparently undergoing change during evolution. Concerning the few examples studied, one cannot exclude the discovery of further intermediate stages.

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