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Copulatory Mechanics in the Funnel-web Spiders *Histoipona torpida* and *Textrix denticulata* (Agelenidae, Araneae)

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Abstract

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The functional morphology of the copulatory organs of *Histoipona torpida* (C. L. Koch, 1834) and *Textrix denticulata* (Olivier, 1789), investigated by semi-thin serial sectioning after freeze-fixation of the animals *in copula*, is described. In *H. torpida*, the long embolus runs through a precisely defined course in the membraneous genital pouch of the female vulva. The patellar apophysis locks the pedipalp in the female epigastric furrow. In *T. denticulata* this is accomplished by the retrolateral tibial apophysis that gets hold at the female scape. In both species the conductor has a locking as well as a guiding function for the embolus. A comparison with previously investigated agelenids shows a surprising diversity in the function of the retrolateral tibial apophysis.

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Introduction

Together with the sheet-web spiders (Linyphiidae), the funnel-web spiders (Agelenidae) present the oldest object of research in spider reproductive biology. It was in 1843 that Menge discovered the mode by which the sperm is transferred from the male genital opening to the actual copulatory organs on the pedipalps. One of his objects was *Agelena labyrinthica*. Since then, a vast number of papers have been published on courtship and copulation habits (Agelenidae: e.g. Montgomery 1903; Osterloh 1922; Gerhardt 1921, 1927, 1933; Gering 1953; Fraser 1987), but little is known about the mechanism of genitalic coupling itself. However, among the rare works on this subject, two outstanding papers deal with funnel-web spiders: *Agelena gracilens* (Osterloh 1922) and *Agelenopsis* spp. (Gering 1953).

The present paper is based on new methods for the investigation of the functional morphology of genitalia: the preparation of serial sections of the genitalia of both sexes fixed in functional contact. It treats another two agelenid spiders which have been considered as members of two additional tribes within the subfamily Ageleninae (Lehtinen 1967), and whose copulatory organs show a striking difference in an important character: in *Textrix denticulata* the sperm transferring appendage, the embolus, is rather short, whereas in *Histoipona torpida* it is extremely long.

Materials and Methods

Penultimate specimens of *Textrix denticulata* (Olivier, 1789) were collected under tree-barks in the Prater-woods

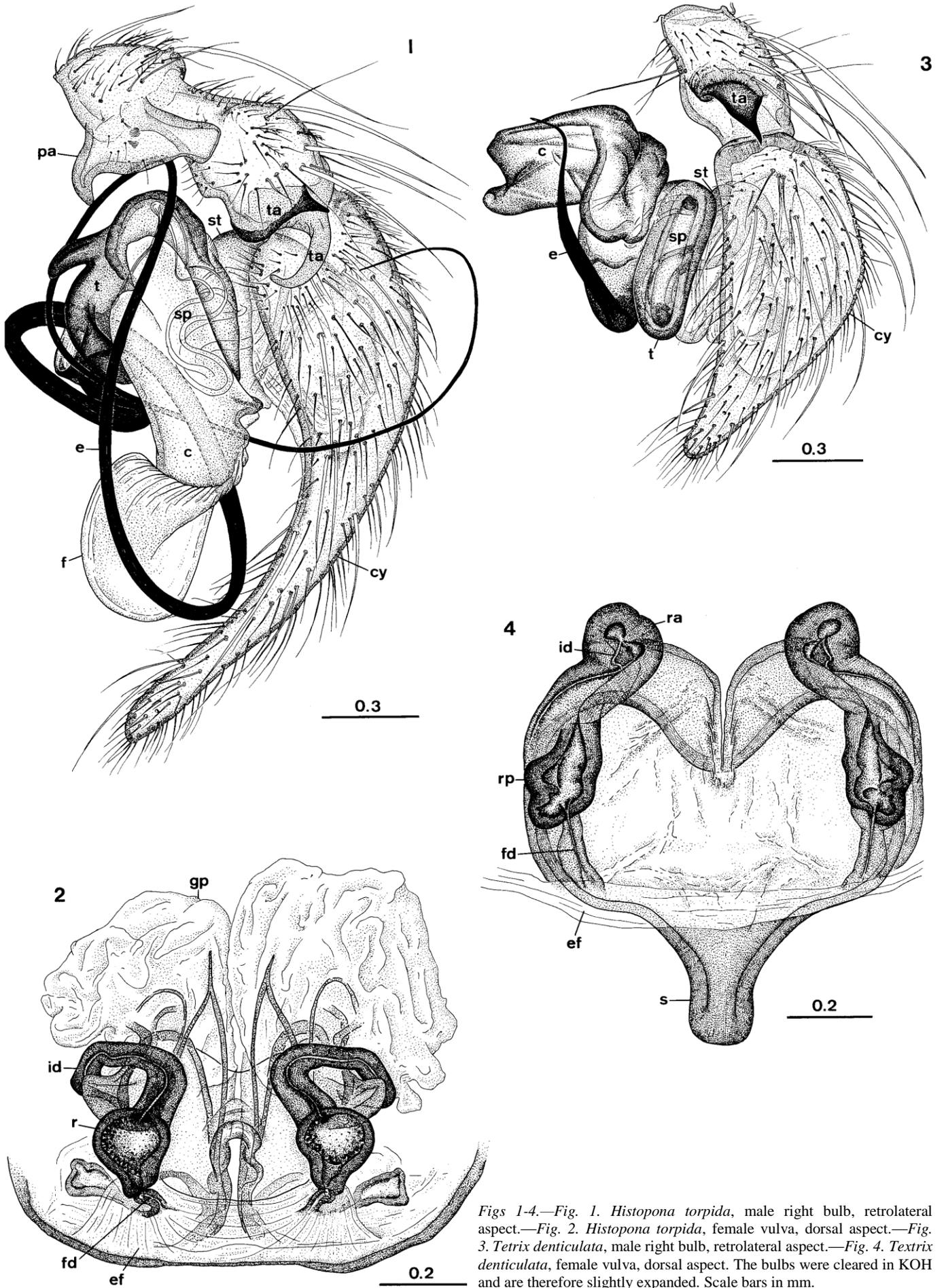
in Vienna, Austria and of *Histoipona torpida* (C. L. Koch, 1834) under stones on the side of a brook in the Mühlviertel near Linz, Upper-Austria. They were reared individually and males were introduced to the females some days after the final moult of both sexes. For freeze-fixation, liquid nitrogen (-196°C) was poured upon the copulating spiders during maximum expansion of the hematodochae. After three weeks in 80% ethanol at -25°C the copulatory organs from three pairs of each species were dissected from the animals, dehydrated and embedded in ERL-4206 epoxy resin under vacuum impregnation. The organs were then serially sectioned (1 µm) with diamond knives on an ultramicrotome (Reichert OmU3). The sections were stained with a mixture of azure 2 (1%) and methylene blue (1%) in an aqueous borax solution (1%) at 80°C for about 10 s. Reconstruction of the serial sections was aided by a computer program (PC-3D).

Classical techniques such as clearing of the genitalia in KOH (10%, 60 min, 80°C) and artificial expansion of the male genital bulb with concentrated lactic acid (15 min, 80°C) were also applied. The copulatory organs were further studied with the scanning electron microscope (Jeol JSM-35CF), both individually and in functional contact (only *H. torpida*). Two copulations of *H. torpida* were observed in the stereomicroscope in order to elucidate the process of insertion.

Results

Morphology of the copulatory organs

The structure of the male as well as the female copulatory organs is in accordance with the general condition in



Figs 1-4.—Fig. 1. *Histopona torpida*, male right bulb, retrolateral aspect.—Fig. 2. *Histopona torpida*, female vulva, dorsal aspect.—Fig. 3. *Tetrix denticulata*, male right bulb, retrolateral aspect.—Fig. 4. *Tetrix denticulata*, female vulva, dorsal aspect. The bulbs were cleared in KOH and are therefore slightly expanded. Scale bars in mm.

entelegyne spiders: the male copulatory organ (Figs 1, 3), the genital bulb, is situated at the distal pedipalpal segment, the cymbium. The membranous tube that connects cymbium and genital bulb is spirally folded at rest and can be highly expanded by a haemolymphatic pressure. It is responsible for the principal movements of the genital bulb during copulation. The basal sclerite of the bulb is the subtegulum, which is distally connected with the tegulum. Two conspicuous appendages arise from the tegulum: the embolus and the conductor. The actual sperm reservoir is the sperm duct, a tube that originates blindly in the subtegulum and passes through the tegulum into the embolus, at whose tip it opens. In both species investigated, the male tibia is provided with sex-specific apophyses (retrolateral tibial apophysis, RTA) and in *Histopona* even the patella shows a prominent branched outgrowth.

The female reproductive organs are situated ventrally on the opisthosoma, anterior to the epigastric furrow. In *Histopona torpida* (Fig. 2), the genital orifices are hidden by a large plate that is connected posteriorly to the ventral body surface (Fig. 6). The genital orifices lead into vast genital pouches that, in rest, present a confusing system of sclerites and folds (Figs 2, 6, 7). These folds, however, are not arranged accidentally as Fig. 2 suggests (this impression is due to the KOH treatment) but in an absolutely constant and orderly manner. Posteriorly, the genital pouches pass into heavily sclerotized tubes that open into the spermathecae. These are provided interiorly with a large number of spines. A short fertilization duct connects the spermathecae with the epigastric furrow.

In *Textrix denticulata* (Fig. 4) a large genital cavity that is divided anteriorly leads immediately into a pair of anterior spermathecae. These are connected with posterior spermathecae which also communicate with the genital cavity and are connected caudally to the epigastric furrow by the fertilization ducts. A heavily sclerotized scape extends backwards over the epigastric furrow (Figs 2, 9).

Genitalia coupling

The mating position of *Histopona* and *Textrix* follows the 'type of the modern hunting spiders' (von Helversen 1976). Consequently, right pedipalp and bulb are locked to the right side of the female epigyne and vice versa.

In both spiders, the male pedipalp is fixed at the female epigyne by a 'fixator'. This structure, however, originates from different segments: in *Histopona* from the patella, in *Textrix* from the tibia. Both branches of the *Histopona* patellar apophysis are engaged into the female epigastric furrow (Figs 6, 10), whereas the tibial apophysis in *Textrix* is locked to the female scape (Figs 9, 11).

A second system of locking is established by the conductor. In *Histopona*, the lamella of the conductor removes the epigyneal plate from the genital orifice by tipping it up anteriorly, and locks near its posterior joint (Figs 5, 6, 10). In *Textrix*, the massive conductor fills up the corresponding genital cavity (Figs 8, 11: right bulb, right cavity). In both spiders the conductor also serves an additional function that is responsible for its name:

it guides the embolus into the small genital orifice. In *Histopona* this is accomplished by the funnel-like part of the conductor (as already pointed out by Gerhardt 1927, and confirmed by own observations) which has no further function, however, as soon as the embolus is driven into the female genital pouch. In *Textrix*, the embolus is guided into the genital opening by a groove on the conductor (Fig. 8).

The actual force that drives the embolus forward is produced by the expanding hematodocha after locking of both the 'fixator' and the conductor. This necessitates a highly flexible zone between conductor and embolus, especially in *Histopona*, where the tegulum must rotate until the embolus is fully inserted. In this species, the complex basis of the embolus and the highly flexible basis of the conductor enable this rotation, whereas in *Textrix* a simple flexible zone between embolus and conductor is sufficient to move the embolus against the conductor the short way to the anterior spermatheca.

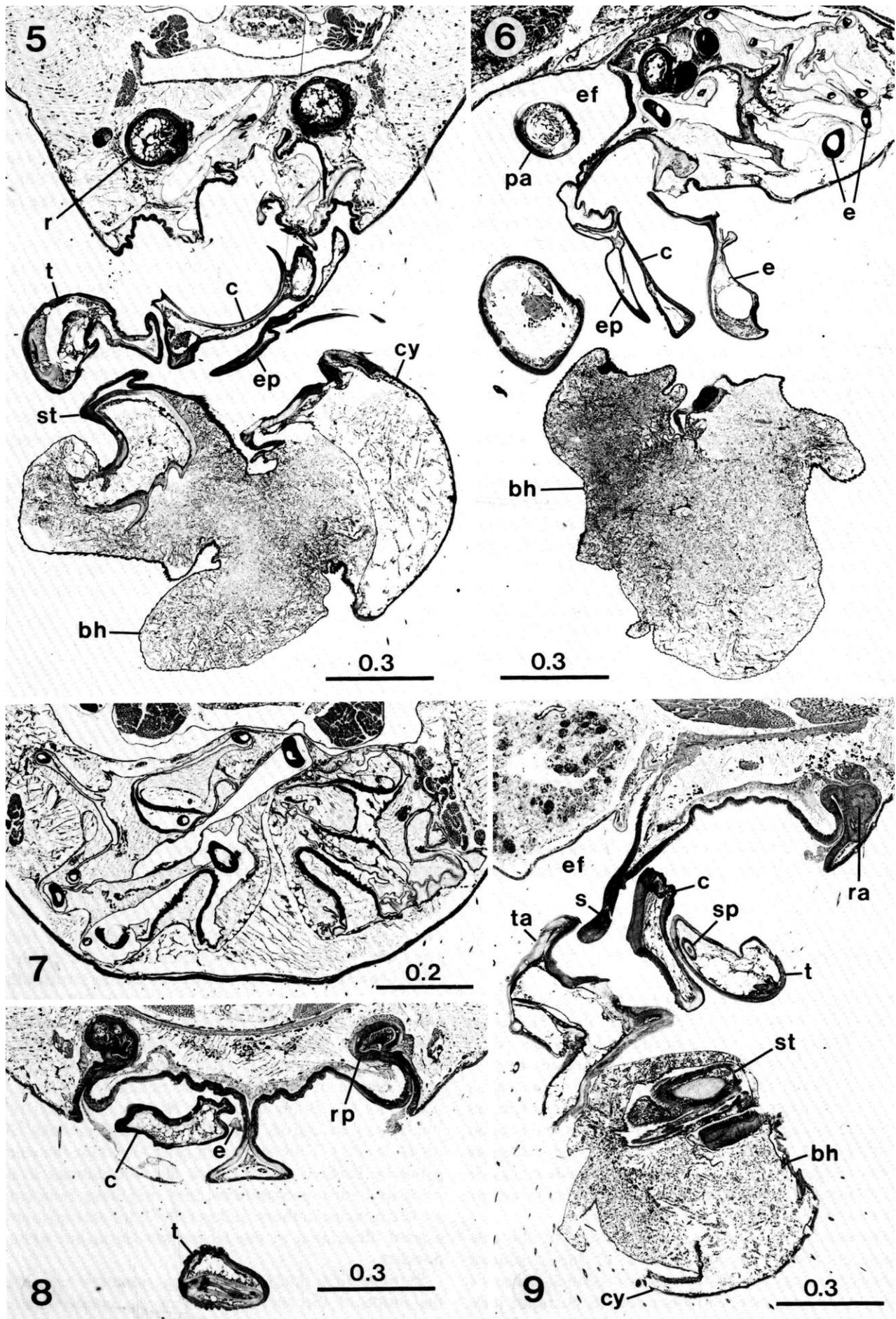
The course of the long *Histopona* embolus in the female genital pouch deserves special attention. The folds of the pouch are highly expanded by the embolus (Fig. 7). The course is absolutely constant (in the three pairs that were serially sectioned) and only this enables the tip of the embolus to find the entrance into the sclerotized tube that leads to the spermatheca.

Discussion

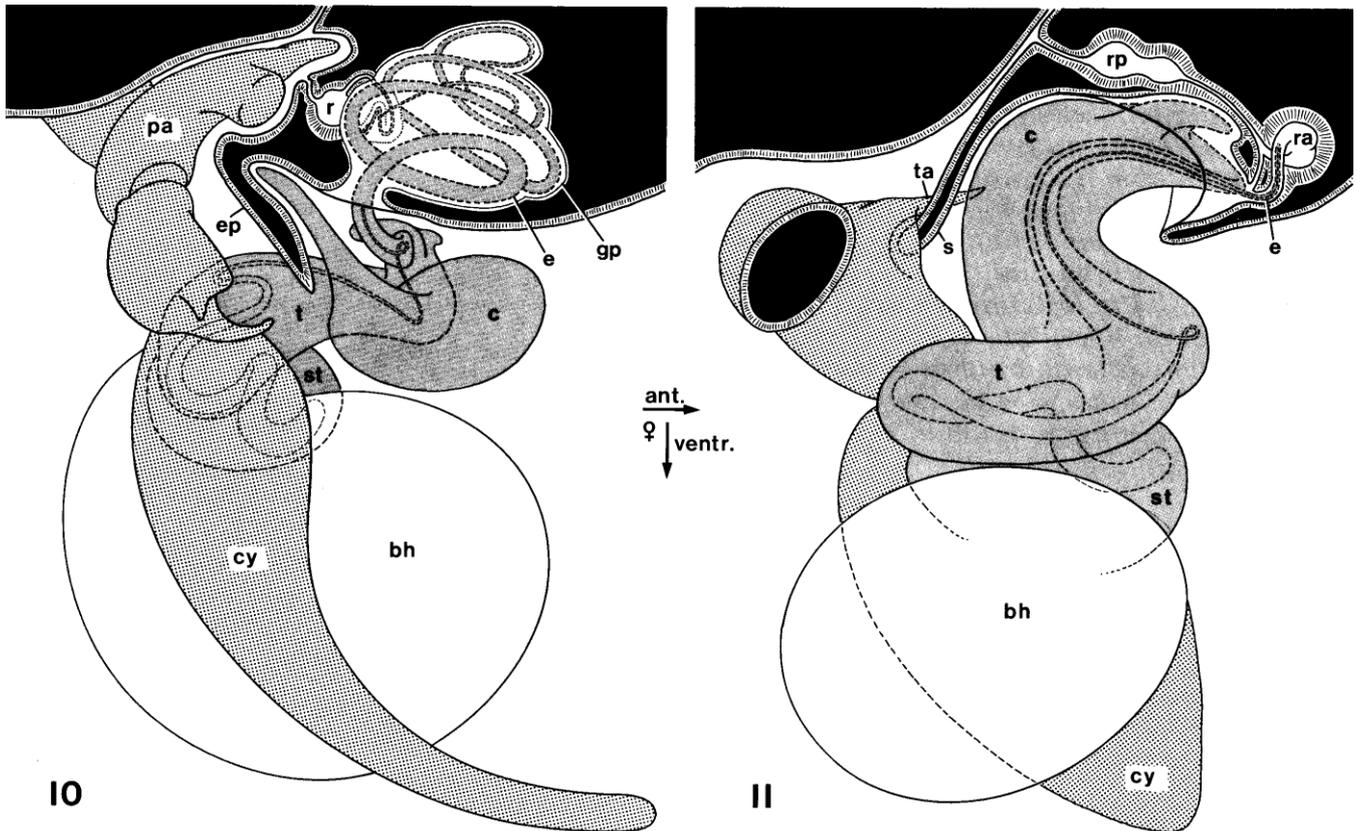
Similarities between the female genitalia in Histopona and Agelenopsis

Female genitalia have until recently been in general severely neglected since the beginning of interest in genital morphology in the last century. Engelhardt's (1910) work is still an outstanding milestone and data for comparative investigation are largely lacking (Carico & Holt 1964; Sierwald 1989). However, the female genitalia of *Histopona* and *Agelenopsis* (see Petrunkevitch 1925; Gering 1953) show some striking similarities. Gering stresses that 'cephalically directed, saccular bursae with definitive plications appear to exist exclusively in the agelenopsids'. The present study shows that this definition exactly matches the condition in *Histopona torpida* as well. In other species of *Histopona*, such pouches also seem to occur (see e.g. Brignoli 1977 for *H. italica*, Brignoli 1972 for *H. hauseri* and *H. myops*; Deeleman-Reinhold 1983 for *H. isolata*). They were even generalized for the whole genus by Brignoli (1980). Since they are difficult to see in cleared vulva-preparations, they may have been overlooked by some authors. Loksa (1969), for example, gives a good illustration of the *H. torpida*-vulva, but without pouches.

Petrunkevitch (1925) found 'long, immobile spines' on the inside of the spermathecae of *Agelenopsis naevia*. Such spines also occur in *Histopona torpida*, and have, to my knowledge, not been mentioned for other agelenid genera. Lehtinen (1967) has placed *Histopona* and *Agelenopsis* into different tribes of the subfamily Ageleninae.



Figs 5-9. Semi-thin sections through the copulatory organs in functional contact.—Figs 5-7. *Histopona torpida*.—Fig. 5. Transverse section through the spermathecae (*r*). The conductor tips up the epigyneal plate.—Fig. 6. Sagittal section. Note the various cuts through the embolus in the genital pouch (only two marked).—Fig. 7. Transverse section through the genital pouches, left side with embolus inserted.—Figs 8,9. *Textrix denticulata*.—Fig. 8. Transverse section, showing the position of conductor and embolus in the genital cavity.—Fig. 9. Sagittal section, showing the correlation between scape and tibial apophysis. Scale bars in mm.



Figs 10, 11. Schematic representation of the coupling mechanics as derived from the serial sections. Females in sagittal section.—Fig. 10. *Histopona torpida*, left bulb applied.—Fig. 11. *Tetrrix denticulata*, right bulb applied.

To evaluate the significance of the above-mentioned vulval structures for phylogenetic analysis, further investigation is needed.

'Luxuries' vs functional structures

Several arachnologists have considered at least some of the various apophyses and appendages of the genital bulb as non-functional luxuries (e.g. Berland 1932). As a general statement, this will never be definitely disproved, but a recent detailed investigation of the cave spider *Nesticus cellulanus* (Huber in press a) has shown that each of the many bulbular structures serve a specific function in copulation. The present paper adds another aspect to this discussion: some structures may have no more function as soon as coupling is achieved. This happens in *H. torpida* with the funnel-like part of the conductor. My own observations are in accordance with Gerhardt (1927), who notes that the embolus is held by this part of the conductor during sperm uptake as well as at the beginning of insertion. Only later, when the embolus is already partially inserted, it springs off from the conductor which now may appear to be a 'functionless luxury'. This may also be true of the tibial apophyses of *H. torpida*. I cannot confirm the view of Gerhardt (1927) that they match exactly into corresponding cavities in the female vulva. Maybe Gerhardt means the patellar apophysis, for he does not mention this structure in his paper. During copulation, however, the tibial apophyses serve no func-

tion. It is assumed, based on the observations, that they play some role in tipping up the vulval plate and inserting conductor and embolus. Thus, at least in *H. torpida*, there is obviously a correlation between the long embolus that must be exactly inserted and the number of structures that appear to be non-functional in coupled organs.

Functional divergence of the retrolateral tibial apophysis

One major obstacle in comparing the functional mechanisms of spider genitalia is the difficulty of identifying the homologous structures (see Coddington 1990). It is not clear, for example, whether the conductors of *H. torpida* and *T. denticulata* are really homologues. An example of a structure that is likely to be homologous throughout a large group of spider families (including Agelenidae) is the retrolateral tibial apophysis (RTA-clade, Coddington & Levi 1991). Some authors have expressed the idea that it might stabilize the genital bulb internally during copulation, but some of these data are based on the examination of artificially expanded, separate male genitalia (e.g. Weiss 1979, 1989; Bennett 1988) and may therefore be doubted in this respect (Huber in press b). Sierwald & Coddington (1988) investigated the expanded genital organ of *Dolomedes tenebrosus* after it had sprung away from the female epigyne. Therefore, this case may not represent the actual situation of the various structures when in contact with the female. A comparison of the functional significance of the pedipalpal apophyses in the

four agelenid species (genera) investigated (*Agelena gracilens*: Osterloh 1922; *Agelenopsis* spp.; Gering 1953; *Histopona torpida* and *Textrix denticulata*: this paper) is therefore especially interesting.

In *Agelena gracilens*, it serves in the internal arrestation of the bulb (Osterloh 1922, p. 348). In *Histopona torpida* its function is unclear but obviously restricted to the process of insertion (see above). The tibial apophysis in *Agelenopsis* 'serves to limit the extent of cymbial flexure' (Gering 1953, p. 13), in *Textrix denticulata* it locks the palp to the female scape. The surprising fact is that the RTA serves four different functions in the four genera investigated. This points to an extremely high functional divergence of homologous structures in the mechanism of coupling.

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Abbreviations Used in the Figures

<i>bh</i>	basal hematodocha
<i>c</i>	conductor
<i>cy</i>	cymbium
<i>e</i>	embolus
<i>ef</i>	epigastric furrow
<i>ep</i>	epigyneal plate
<i>f</i>	funnel-like part of the conductor
<i>fd</i>	fertilization duct
<i>gp</i>	genial pouch
<i>id</i>	insemination duct
<i>pa</i>	patellar apophysis
<i>r</i>	receptacle (spermatheca)
<i>ra</i>	anterior receptacle (spermatheca)
<i>rp</i>	posterior receptacle (spermatheca)
<i>s</i>	scape
<i>sp</i>	sperm duct in the male genital bulb
<i>st</i>	subtegulum
<i>t</i>	tegulum
<i>ta</i>	retrolateral tibial apophysis (RTA)

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