

# Genital mechanics and sexual selection in the spider *Nesticus cellulanus* (Araneae: Nesticidae)

BERNHARD A. HUBER

Institut für Zoologie der Universität Wien, Althanstraße 14, A-1090 Wien, Austria

Received May 17, 1993

Accepted September 24, 1993

HUBER, B.A. 1993. Genital mechanics and sexual selection in the spider *Nesticus cellulanus* (Araneae: Nesticidae). Can. J. Zool. 71: 2437–2447.

The spider *Nesticus cellulanus* (Clerck, 1758) was investigated with respect to the functional morphology of its genital organs, using for the first time the method of freeze-fixation of the spiders in copula by liquid nitrogen and subsequent preparation of serial sections of the copulatory organs in functional contact. Use of this method provides new and detailed insights into the function of complex genitalia. Previous ideas, based on artificial expansion of the male copulatory bulbs and simulation experiments, must be rejected or doubted. The role of female choice as a universal cause of species specificity and complexity in genitalia, proposed by Eberhard, is discussed. The total absence of sensilla in the contact zones of the female copulatory organs indicates that the female is not stimulated by the copulatory organs of the male, nor can she evaluate their mechanical fit. "Female choice" can therefore act only indirectly, by influencing the number of offspring, which is assumed to be correlated with the fitting of the male genitalia, thus determining the number of sperm transferred. This postmating "choice" is absolutely independent of the female nervous system and is seen to be at least an important additional mechanism of sexual selection.

HUBER, B.A. 1993. Genital mechanics and sexual selection in the spider *Nesticus cellulanus* (Araneae: Nesticidae). Can. J. Zool. 71: 2437–2447.

La morphologie fonctionnelle des organes génitaux a été étudiée chez l'araignée *Nesticus cellulanus* (Clerck, 1758). La méthode de fixation par congélation des animaux in copula au moyen d'azote liquide et la préparation subséquente de coupes sériées des organes copulateurs au moment d'un contact fonctionnel ont été utilisées pour la première fois chez des araignées. Cette méthode permet l'investigation plus poussée et plus détaillée du fonctionnement d'organes génitaux complexes. Les concepts antérieurs, basés sur le gonflement artificiel des bulbes copulateurs mâles et sur des expériences de simulation, doivent être abandonnés ou au moins mis en doute. L'hypothèse du choix de la femelle comme cause universelle de la spécificité à l'espèce et de la complexité des organes génitaux, telle que proposée par Eberhard en 1985, est examinée. L'absence totale de sensilles dans les zones de contact de ses organes génitaux indique que la femelle n'est ni stimulée par les organes copulateurs du mâle, ni capable d'estimer leur possibilité d'ajustement mécanique. Le « choix de la femelle » ne joue donc qu'un rôle indirect, en influençant le nombre de rejetons présumé en corrélation avec l'ajustement des organes génitaux du mâle, déterminant ainsi la quantité de sperme transférée. Ce « choix » post-copulatoire est absolument indépendant du système nerveux de la femelle et est considéré au moins comme un mécanisme supplémentaire important de la sélection sexuelle.

[Traduit par la rédaction]

## Introduction

Owing to their great value in species identification, the copulatory organs of spiders have been intensively studied since the 19th century. Most papers have been limited to descriptions of the various structures and appendages, especially of the male copulatory organ, the genital bulb. In the last few decades, increasing numbers of authors have become interested in the exact functioning of these sometimes extremely complex organs (for references see below) as well as in the evolutionary factors that could have brought about complexity and species specificity in genital organs (for a review see Eberhard 1985).

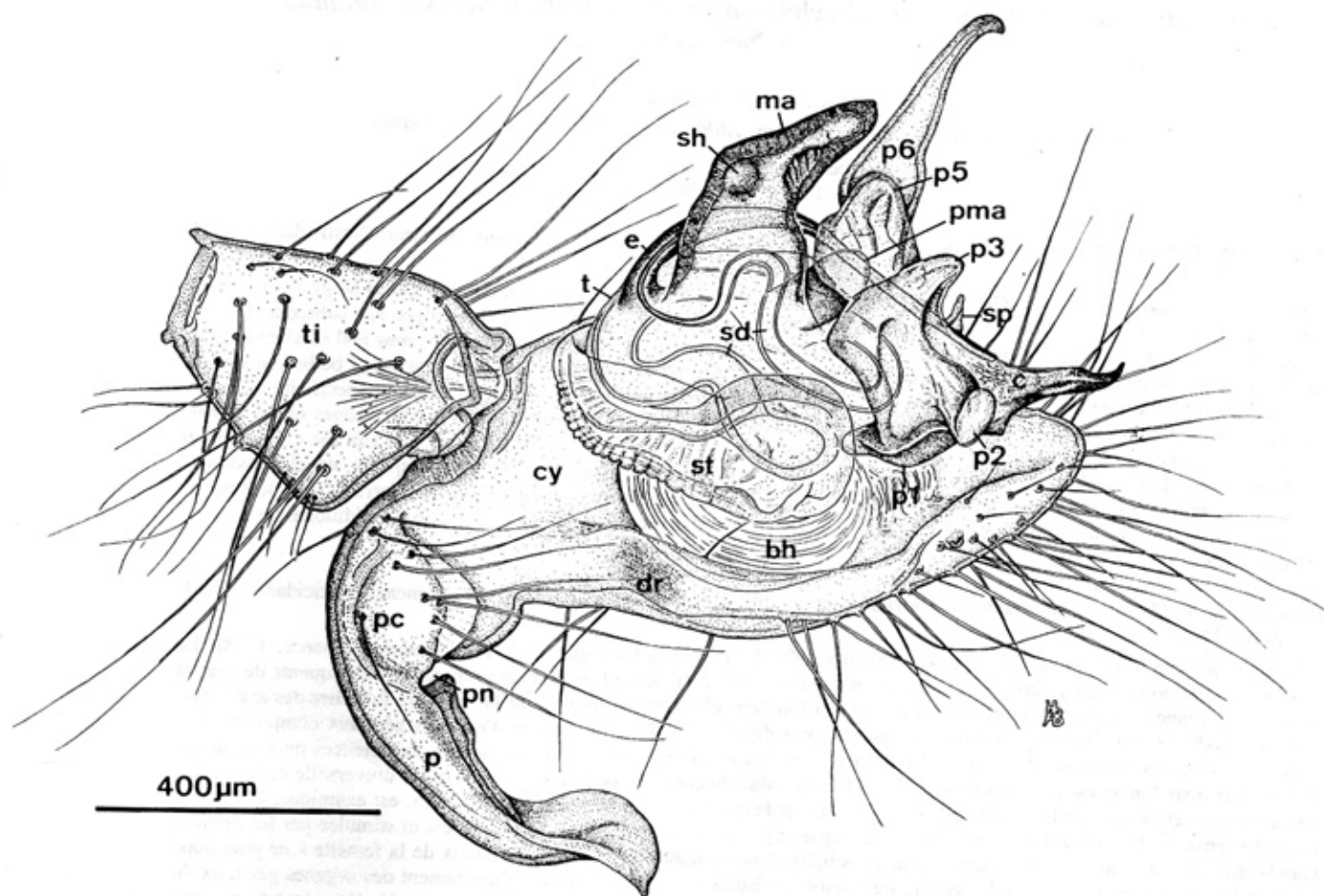
Most authors who have gone beyond mere description have inferred the function from examination of the separate male and female genitalia (e.g., Gering 1953; Weiss 1979, 1981, 1982, 1983, 1989; Martin 1981; Weiss and Heimer 1982; Loebricks 1984). Only a few arachnologists have succeeded in fixing spider pairs in copula (Osterloh 1922; Melchers 1963; Cooke 1966; Grasshoff 1968, 1973; van Helsdingen 1965, 1969). However, serial sections of the organs in functional contact have never been made. This technique permits detailed and specific statements to be made about the actual mechanical fitting together of the various structures. The combination of freeze-fixation and preparation of serial sections promises to give new and more detailed insights into the functional morphology of spider genitalia.

The most accepted theory to explain the complexity and species specificity of the genitalia was proposed by Eberhard (1985). He assumed that the genitalia of the male may function as "internal courtship" devices that stimulate the female during copulation, or may be used by the female as a criterion of choice by testing their mechanical fit. Detailed examination of sexual behaviour, together with genital mechanics and the morphology of the contact zones, should help to evaluate Eberhard's theory, as well as the other theories that have been proposed (see Shapiro and Porter 1989; Mayr 1963; Kraus 1968; Grasshoff 1975).

*Nesticus cellulanus* is a more or less troglomorphic, web-weaving spider exhibiting the rare phenomenon of perennial occurrence of adult (and penultimate stage) specimens (Tretzel 1954). It is easy to rear, and copulations are readily obtained (Gerhardt 1927). These qualities make *N. cellulanus* very suited to a lengthy and thorough investigation of sexual behaviour and genital mechanics.

## Materials and methods

Penultimate-stage specimens of *Nesticus cellulanus* (Clerck, 1758) were collected from a heap of paving stones, a wine cellar, and the bank of a brook in the Mühlviertel near Linz, Upper Austria. They were housed in individual circular clear plastic containers (7 cm diameter, 3 cm depth) with a mixture of gypseous alabaster and



ABBREVIATIONS: *ad*, anterior diverticulum of the vulval pocket; *ant*, anterior; *bh*, basal hematodocha; *c*, conductor; *co*, copulatory orifice; *cy*, cymbium; *dr*, dorsal ridge of the cymbium; *e*, embolus; *ef*, epigastric furrow; *efs*, epigastric furrow sclerite; *fd*, fertilization duct; *id*, insemination duct; *ma*, median apophysis; *p*, paracymbium; *pc*, paracymbial cavity; *pma*, paramedian apophysis; *pn*, paracymbial notch; *p1*–*p6*, processes of the conductor complex; *s*, spermatheca; *sd*, sperm duct in the male bulb; *sh*, subapical hump of the median apophysis; *sp*, subapical projection of the embolus; *st*, subtegulum; *t*, tegulum; *ti*, tibia; *vf*, ventral fold in the female vulva; *vpl*, vulval pocket, lateral part; *vpm*, vulval pocket, medial part.

FIG. 1. Left genital bulb of male *Nesticus cellulanus*, with cymbium and tibia. As a result of treatment with KOH, the basal hematodocha is slightly expanded and the embolus has fallen out of its position at rest (cf. Fig. 3c).

activated carbon (2:1) in the bottom. The spiders were fed with *Drosophila* at the rate of one every 2 or 3 days.

Some copulations, including courtship and precopulation behaviour, were continuously observed with a stereomicroscope and recorded. Additional pairs were freeze-fixed in copula with liquid nitrogen ( $-196^{\circ}\text{C}$ ), by either immersing the spiders with their container into the fluid or by pouring the fluid over them. They were quickly transferred to cold ( $-25^{\circ}\text{C}$ ) alcohol (70%) or Carnoy's solution (Romeis 1989) and kept in these fixatives at  $-25^{\circ}\text{C}$  for about 1 month. The copulatory organs of three of these pairs were then dissected from the animals, dehydrated, and embedded in ERL-4206 epoxy resin (Spurr's medium) under vacuum impregnation. The organs in functional contact could then be serially sectioned ( $1\ \mu\text{m}$ ) with an ultramicrotome (Reichert OmU3), using glass or diamond knives. The sections were stained with a mixture of azur II (1%) and methylene blue (1%) in an aqueous borax solution (1%) at  $80^{\circ}\text{C}$  for about 15 s.

Because nitrogen fixation caused some damage to the soft tissues, additional serial sections were made of the separate copulatory organs which had been fixed in Duboscq–Brasil solution (Romeis 1989). A computer program (PC-3D) helped in the three-dimensional reconstruction of the serial sections.

Numerous classical techniques were also used to study the morphology of the copulatory organs. Bulbs and vulvae were treated with

KOH and lactic acid at various concentrations and temperatures. Artificial expansion was studied using fresh bulbs, which were put into concentrated lactic acid (15 min,  $80^{\circ}\text{C}$ ) and subsequently transferred to distilled water. The organs were further studied with a scanning electron microscope (Jeol JSM-35CF), both individually and in functional contact.

## Results

### Male copulatory organs

In spiders the male copulatory organ, the genital bulb, is situated on the distal segment of the pedipalpus. This segment is called the cymbium, and in *N. cellulanus*, as in other nesticids, it is the only pedipalp segment that is significantly modified. It bears a prominent outgrowth, the paracymbium (*p* in Fig. 1), which is distally flattened and proximally provided with a notch and a cavity. In contrast to the genital bulb, the cymbium is covered with hair sensilla. Only the distal part of the paracymbium is free of hairs.

The genital bulb is connected to the cymbium by an inflatable membraneous saclike structure, the basal hematodocha, which is spirally folded at rest. This results in rotation of the bulb when the basal hematodocha is expanded (see Fig. 7b).

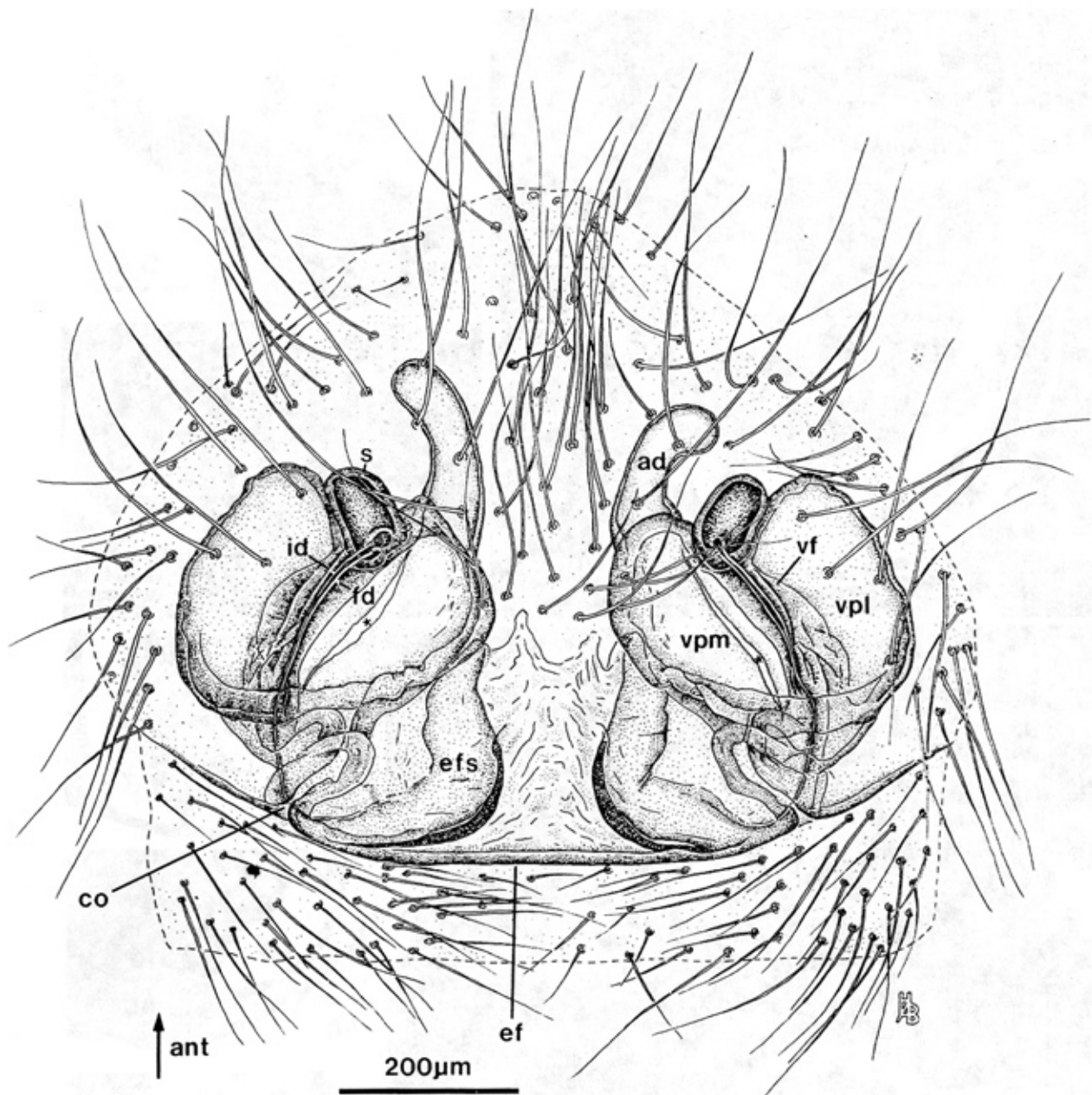


FIG. 2. Vulva of female *Nesticus cellulanus* after KOH treatment, ventral view. An asterisk marks the crevice in the female cuticle caused by the male median apophysis during copulation (cf. Fig. 6a).

A small strip of this sac is more thickly sclerotized. This petiolus forms an elastic joint between the cymbium and the subtegulum. The subtegulum, the basal part of the genital bulb, is a ringlike sclerite which is distally connected to a second ringlike sclerite, the tegulum. On one side, these sclerites are membranously linked by the median hematodocha, which is not spirally folded.

Several apophyses arise from the tegulum (Figs. 1, 3): (i) the embolus, by which the sperm duct leaves the tegulum. The sperm duct, which has its origin in the subtegulum, opens at the tip of a subapical projection of the embolus; (ii) the median apophysis, a thickly sclerotized outgrowth of the tegulum, which bears a subapical hump; (iii) the paramedian apophysis, a simple knob-like structure; and (iv) the conductor complex, the most complex structure in the *N. cellulanus* bulb, which is linked to the tegulum by a flexible stalk. It may be divided into two parts, which are flexibly connected. Part A

bears the actual (functional) conductor and four processes, which will be referred to as *p1*–*p4*. Process *p4* grips the embolus when at rest (Fig. 3c). Part B consists of another two processes, *p5* and *p6*. These structures will be described in more detail in the context of their function during copulation.

#### Female copulatory organs

In female *N. cellulanus*, only a simple sclerotized plate, the epigyne, marks the position of the copulatory organs ventrally on the opisthosoma, anterior to the epigastric furrow. The small copulatory orifices (*co* in Fig. 2) open into large vulval pockets, which comprise three major structures: a lateral part and a medial part, which are separated by a ventral fold (Figs. 4b–4d), and an anterior diverticulum of the medial part. On the ventral folds, small insemination ducts have their origin (Fig. 4d). They make their way through these folds to the thickly sclerotized spermathecae. The fertilization ducts origi-



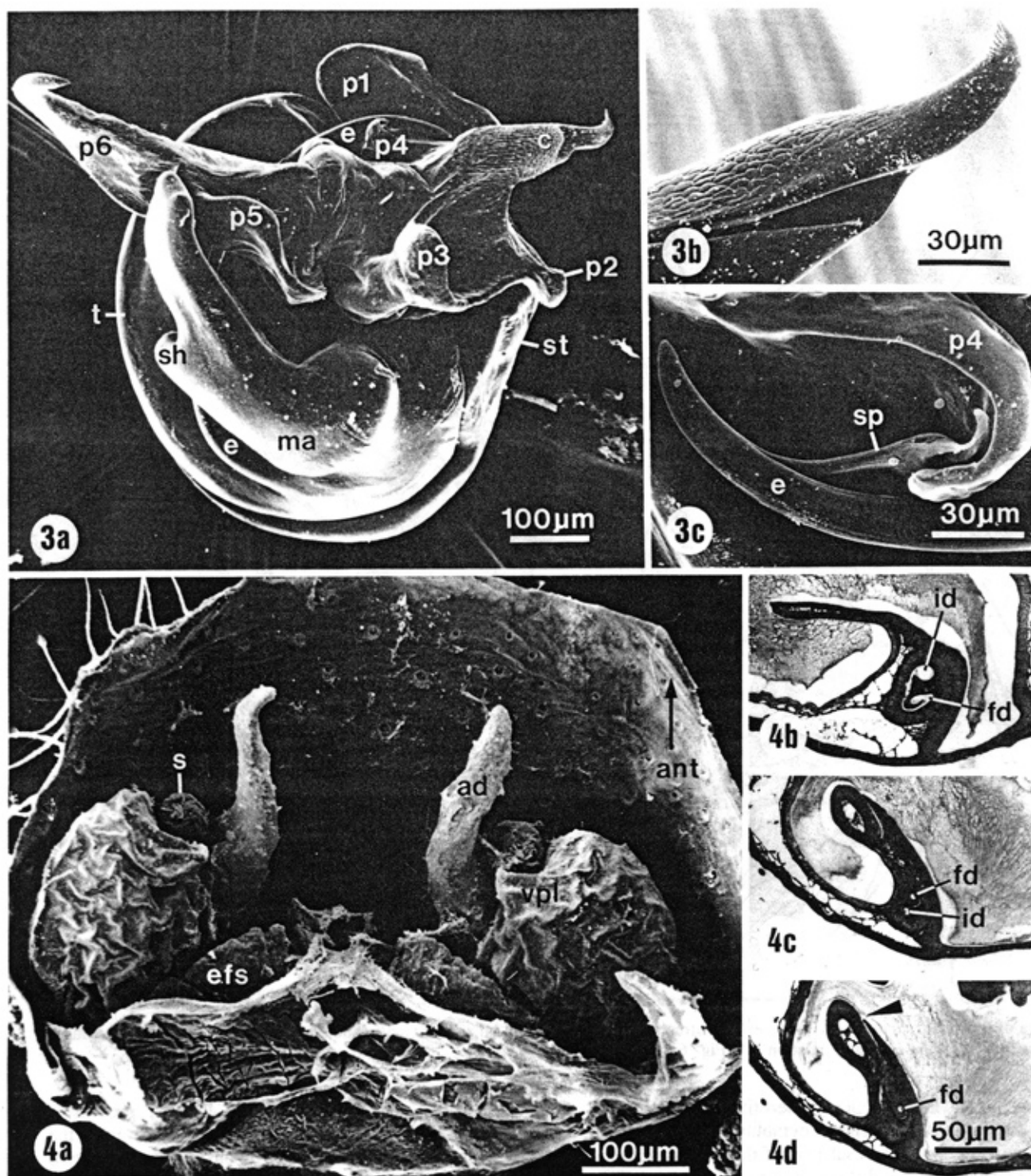


FIG. 3. SEM micrographs of the male genital bulb. (a) Genital bulb without cymbium. (b) Conductor with embolic groove. (c) Tip of embolus, locked into process *p4* of the conductor complex. FIG. 4. Female vulva. (a) SEM micrograph after KOH treatment, dorsal view. (b–d) Cross sections through the ventral fold, showing the division of insemination and fertilization ducts; *b* is a section taken close to the spermatheca and *d* shows the opening of the insemination duct (arrowhead).

nate in the spermathecae immediately adjacent to the insemination ducts, lead backwards parallel to these, then bend medially before opening into the epigastric furrow. Insemination and fertilization ducts on each side are derived from a single duct which is divided into two longitudinally (Figs. 4b, 4c).

Glands can be found in association with both the spermathecae and the anterior diverticula. Hair and slit sensilla are absent in a large area around the copulatory orifices as well as in the epigastric furrow. Scanning electron microscopy revealed a large number of small pores on the back side of the epigastric

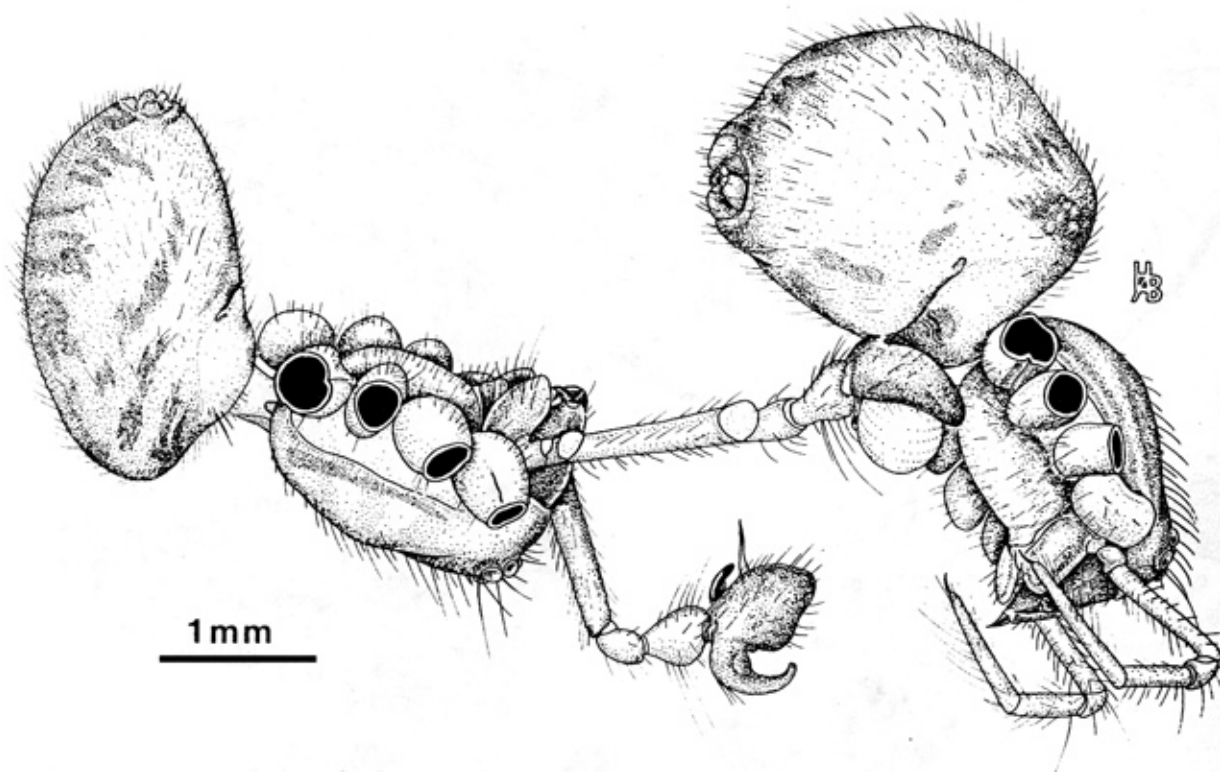


FIG. 5. Copulatory position in *Nesticus cellulanus*. The legs are omitted because they have no special function during copulation.

furrow. They are either isolated or grouped into pore fields. The anterior side of the epigastric furrow is provided with two strongly sclerotized plates, the epigastric furrow sclerites.

#### Sexual behaviour

Out of 22 copulations, 5 were continuously observed under the stereomicroscope. Courtship, copulation, and postcopulation behaviour were recorded. All observations were made at room temperature. No differences could be found between copulations during bright daylight and in dim artificial light. The males were introduced to the females 3 days to 3 weeks after the final moult of both sexes.

On coming into contact with the female's silk thread, the male soon commences courtship, which initially consists of very gentle knocking with the palps and jerking with the opisthosoma. These two elements are always present and increase in intensity until copulation takes place. Other elements were seen only sporadically (observations included 17 additional copulations, which were interrupted either by fixation or for other experiments), and these consist of the following: waving of the front legs, partial destruction of the female web, and a gentle seesawing motion with the whole body.

Courtship finally results in the female turning towards the male and suspending herself in a more vertical position. The male now approaches the female, knocking strongly with the palps, and extends one of the palps with the paracymbium rotated from a dorsally to a medially directed position. In this position the conductor is pushed into the genital orifice of the female. In most cases observed, the male missed the orifice in up to 10 trials before insertion was successful. Full anchoring of the bulb in the female's epigyne (described later) results in the copulatory position shown in Fig. 5.

Courtship duration (from transfer of the male to the female container until insertion of the bulb) ranged from 1.5 to 47 min ( $\bar{x}$  = 18.6, SD = 12.6;  $N$  = 21). Mean duration of the first insertion was 9.9 min (SD = 1.7;  $N$  = 12).

The basal hematodocha is rhythmically expanded and partially collapsed, full expansion being produced at intervals ranging from 1 s at the beginning of insertion to more than 10 s towards the end. The time of full expansion also increases from 1 to about 8 s. Extraction of the bulb is initiated by the male but may sometimes be forced by some movement of the female.

The second bulb is inserted after a short period of rest and renewed palpal knocking, in most cases without separation of the animals. A second insertion was only observed five times ( $\bar{x}$  = 17.3 min, SD = 4.3) and lasted significantly longer ( $t$  test,  $p$  < 0.0001).

After extraction of the second bulb, the spiders separate peaceably (sometimes the female clamps a male foreleg between her chelicerae for some seconds) and both engage in cleaning their copulatory organs before coming to rest after a few minutes to 1 h. The movements of the male now lead to abrupt rushes by the female towards him. No further insertions were ever observed, nor did a female ever prey upon the male. The animals were separated about 2 h after finishing copulation.

Two forms of rejection behaviour by the female were observed: newly moulted or "unwilling" virgin females simply do not react to male courtship, and inseminated females react "aggressively" as described above. The five inseminated females from the continuously observed copulations were confronted with virgin males at intervals of a few days until 3 weeks after oviposition, but none accepted a second mate.

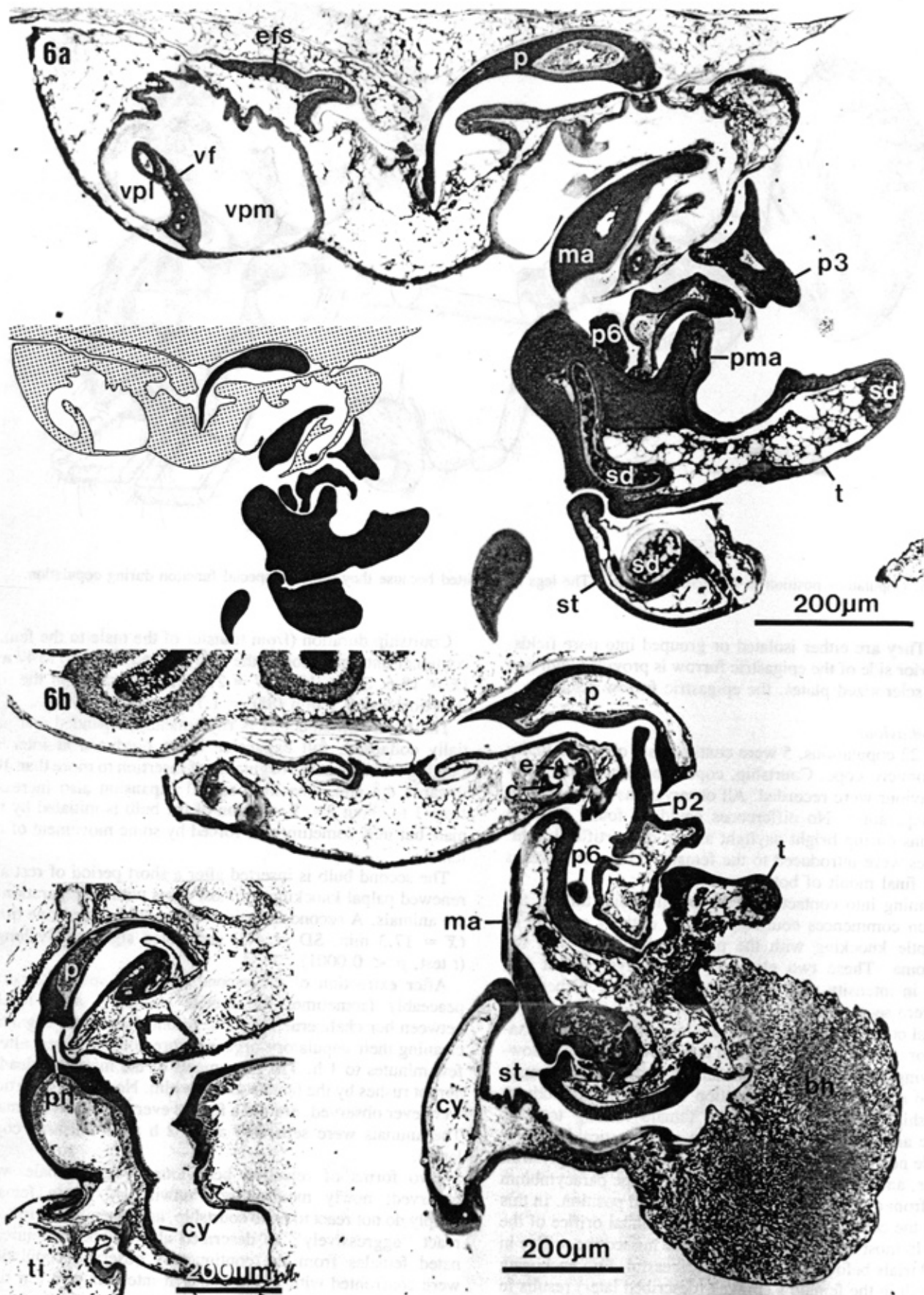


FIG. 6. Cross sections (of the female) through the genital organs in functional contact. (a) Note the position of the paracymbium and median apophysis. On the nonfunctional side the cuticula of the vulval pocket is still intact. Inset: Graphic representation of male (stippled) and female (solid) structures. (b) The same, farther back. Inset: Female in sagittal section, with the paracymbium hooked into the epigastric furrow.



To ascertain that males can inseminate more than one female, one male was allowed to copulate with two females ( $f1$  and  $f2$ ), one 3 days after the other. Eggs were laid after 54 ( $f1$ ) and 37 ( $f2$ ) days, and 51 ( $f1$ ) and 34 ( $f2$ ) spiderlings hatched after 25 ( $f1$ ) and 28 ( $f2$ ) days.

Five pairs were separated after the first bulb was inserted. When a second male was introduced within 1 h, he was able to insert both bulbs. The females showed no rejection behaviour ( $N = 3$ ). In the two other cases the second male was introduced after 2 days. The females seemed more aggressive than virgin females, nevertheless the males managed to insert one bulb (the one corresponding to the "virgin side" of the female). They were not able to insert the other bulb, even after dozens of trials. However, I could not find any mating plug in serial sections of two "half-virgin" vulvae.

An interesting but singular observation should be briefly noted: one male who had lost one cymbium in his final moult managed to insert the intact bulb and then tried to insert the other (non-existent) one for more than 2 h. After he had boxed his stump hundreds of times against the female's epigyne, the pair was separated.

#### Genital mechanics

The complicated succession of insertion, expansion, and locking of the bulb into the female can be inferred from observations of the process under the stereomicroscope and by analysis of the copulatory organs before and after locking.

The first part of the male's bulb to be locked into the female is the conductor. It has to be pushed through the small genital orifice into the female vulval pocket (left bulb into left vulva). The following three steps are all caused by the expansion of the basal hematodocha and therefore occur more or less simultaneously: (i) the paracymbium is slipped into the epigastric furrow and locked into the conductor complex; (ii) the median apophysis is pressed against process  $p6$  of the conductor complex and pierces the thin cuticular ventral wall of the female's vulval pocket (Fig. 6a); (iii) the embolus is driven through the conductor into the female's vulval pocket.

At this point full locking is attained. The pulsating movements that occur during expansion have at least two consequences: the tegulum and the conductor complex are pressed more firmly against the female's epigyne (especially by the median hematodocha, which is only expanded during phases of high pressure), and the embolus is driven deeper into the female.

To sum up, we can discern 11 points of locking and contact (Figs. 6, 7):

1. The paracymbium is locked into the female epigastric furrow. Slipping back is prevented by the paracymbial notch, which is hooked into a cuticular fold in the epigastric furrow (Fig. 6b, inset).

2. The conductor is hooked into the genital orifice of the female. The grooves on the inner side of its apical hook (Fig. 3b) increase the frictional resistance.

- 3–5. Part A of the conductor complex is further locked into the paracymbium by processes  $p1$  and  $p2$  ( $p2$  is pressed into the paracymbial cavity; Figs. 7a, 7b), and is pressed against the lateral surface of the epigyne with the flat process  $p3$ .

6. Likewise, process  $p5$  of the conductor complex is pressed against the frontal surface of the epigyne, which may become deeply dented in this region.

7. The long, tapering process  $p6$  is squeezed between the median apophysis and the conductor complex. The elasticity of the cuticular joint of this apophysis serves to force back

the tegulum when expansion (Fig. 7b) of the hematodochae decreases.

- 8–10. The median apophysis is locked into the median part of the vulval pocket of the female. The subapical hump regulates the degree of insertion, and basally the median apophysis is supported by the dorsal ridge of the cymbium (Fig. 6b).

11. The paramedian apophysis stops the rotation of the tegulum when it is pressed against the fixed conductor complex.

## Discussion

#### Methodological aspects

Heimer (1982), Weiss and Heimer (1982), and Weiss (1981, 1983) have published a series of papers dealing with the functional morphology of the genitalia in certain nesticid species. Their work is based on the study of the separate genital organs and artificial expansion of the male bulb. Their results contradict those presented in this paper in nearly every detail. The reason is that during copulation, the female copulatory organ forces the various male parts in specific directions. This is not the case during artificial expansion.

A great number of authors have studied genital mechanics using the same methods (e.g., Gering 1953; Bhatnagar and Rempel 1962; Bhatnagar et al. 1971; Sadana 1972; Loerbroks 1983, 1984; Martin 1981; Schult and Sellenschlo 1983). The present study confirms the view that complex genitalia can only be understood by observing them as functional units (Grasshoff 1975). Artificial expansion may be of great value for taxonomists (Shear 1967), but, at least in *N. cellulanus* and other spiders examined (B.A. Huber, in preparation), it produces an incorrect picture of natural expansion during copulation.

#### Morphological and functional aspects

New terms have been introduced in this paper for various parts of the male bulb and the female vulva. Terminological problems arise from two sources: (i) the names of particular structures should not be contradictory to their function, and (ii) homologous structures, and these alone, should have the same name. In this paper only the functional aspect can be discussed adequately. So the names are not to be regarded as indicating homologies. This is especially true for the following structures:

The "paracymbium" of the Nesticidae may not be homologous with the paracymbium of the Linyphiidae and Erigonidae (Lehtinen and Saaristo 1980). The nesticid "median apophysis" may be homologous with the "theridiid tegular apophysis" (Coddington 1990). This view is strengthened by the fact that the sperm duct makes a loop into the nesticid "median apophysis" (Fig. 1), as is also true of the "theridiid tegular apophysis." Coddington (1990) further speculated that the actual median apophysis of other orb-weaving groups might be represented in Nesticidae by the "terminal apophysis," which is called process  $p6$  in this paper. Since the actual median apophysis and the actual conductor arise ontogenetically from the same (distal) portion of the dorsal lobe of the claw fundamen (Bhatnagar and Rempel 1962), it seems probable that they correspond to "part A" and "part B" of the "conductor complex." So the conductor complex could turn out to be a derivative of one lobe fragment that was not divided by truncation of the developmental process (cf. Coddington 1990, p. 9).

Similarly confusing is the situation in the female's genitalia. No broad comparative studies have been carried out since Engelhardt (1910), and the terms used can only be of func-

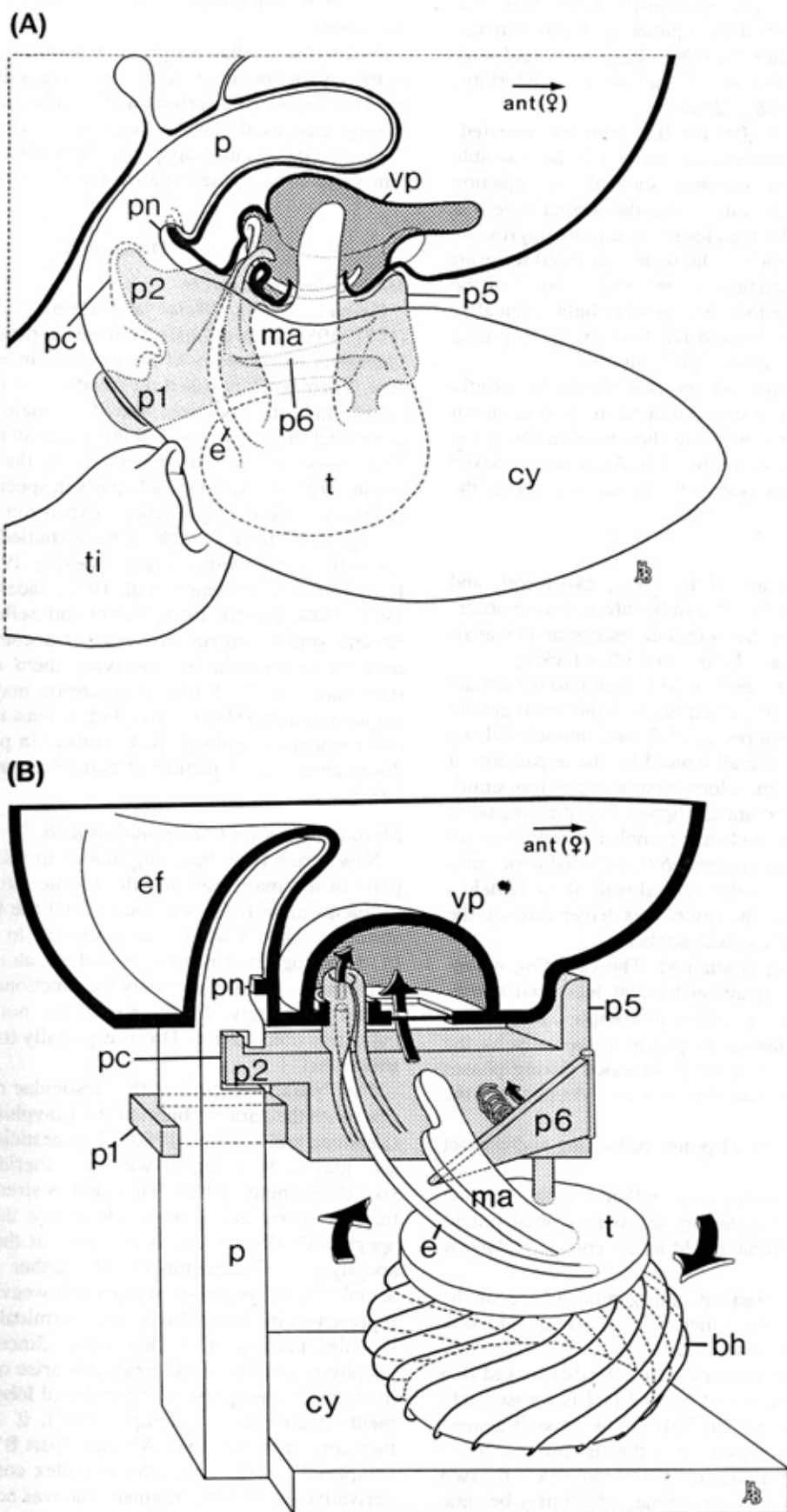


FIG. 7. The copulatory organs (left vulva, left bulb) in functional contact. The finely stippled area is the conductor complex. (a) Schematic representation of the main locking mechanisms. The subtegulum and hematodochae are omitted. (b) Schematic diagram showing the principal movements caused by expansion of the basal hematodocha. Note that the cymbium and conductor complex are already fixed.



tional significance. The "vulval pockets" in this paper correspond to the "réservoirs spermatiques" of Dumitrescu (1979, 1980). Since they do not seem to store sperm, but serve to hold the median apophysis of the male during copulation, the neutral term (after Gertsch 1984) is preferred. However, the presence of anterior diverticula with glands indicates that there are additional, undetermined functions.

Dumitrescu (1979, 1980) has further misinterpreted the function of the vulval sperm ducts, as Weiss (1981) has already pointed out.

Weiss (1981, 1982) assumes that a membranous tube might be evaginated from the embolus to reach the spermatheca. I could not find any structure that could support this speculation. Rather, the transport of sperm in female *N. cellulanus*, not only from the spermathecae to the fecundation site (i.e., uterus externus) (Lopez 1987) but also to the spermathecae, seems to be female controlled, because the embolus (diameter 17  $\mu\text{m}$ ) cannot possibly pass through the insemination duct (diameter 6  $\mu\text{m}$ ).

#### Behavioural aspects

The most intriguing aspect of the results concerning behaviour is the difference from those obtained by Gerhardt (1927) with the same species in Thuringia, Germany. Some of the differences in courtship behaviour may be due partly to the difficulty of describing certain movements with our vocabulary, but the insertion pattern is absolutely different. Gerhardt observed four to six insertions in all pairs with virgin females. The whole copulation lasted "nur wenige Minuten," which may mean 5 to a maximum of 10 min in Gerhardt's terms. He observed eight copulations and states that all were of the same pattern and duration. In each of the five copulations that were continuously observed in this study, there were only two insertions: copulations lasted from 23 to 35 min.

The differences could be attributed to different housing conditions. However, this seems very unlikely for several reasons. Gerhardt's glass vessels were not very different from the plastic boxes used in this study, and the only conditions he specifically provided were humidity and food. In general, copulation duration may vary with greatly varying temperatures (Costa and Sotelo 1984), but courtship and insertion patterns are considered to be highly consistent within a given species (e.g., Rovner 1972, 1973, 1974; Uetz and Denterlein 1979; Starr 1988; but see Jackson 1980).

Therefore the results indicate unusual differences in copulatory behaviour between the Thuringian and the Austrian populations. But only a comparative investigation under identical conditions and using crossing experiments could justify division of *Nesticus cellulanus*, the type species of the Nesticidae.

#### Species specificity and complexity in spider genitalia: the result of female choice?

Several theories have been proposed to explain species specificity and complexity in animal genitalia. The oldest is probably the lock and key hypothesis, which was proposed about 150 years ago for insects and has fallen into disrepute for several reasons (see Eberhard 1985; Shapiro and Porter 1989). The same is true of the pleiotropism hypothesis of Mayr (1963) (see Eberhard 1985). The most satisfying theory seems to be the female choice hypothesis, which is based on Darwin's (1871) ideas on sexual selection and was proposed by Eberhard in 1985. This hypothesis assumes two types of criteria that may influence the female in her choice of mates

and (or) sperm. First, sensory stimuli that are brought about by the male's copulatory organs, and secondly, their "mechanical fit." Unfortunately, Eberhard (1985) did not define female choice, but it is obvious from many passages of his text that he means some sort of differential behavioural or physiological responsiveness by the female in response to male traits. In one passage (Eberhard 1985, p. 76) he mentions a sort of female choice ("mechanical discrimination") that is not correlated with any sensory reception by the female's nervous system (in *Pedetes surdaster*, where the male's penis mechanically opens a valve in the female's vagina during copulation). This sort of "choice" should probably be considered to be much like that of an artificial lock, which "decides" whether the key may enter or not, with the difference that a suboptimal key is not excluded but its use leads to a poorer outcome (e.g., fewer sperm are transferred), and use of a supraoptimal key to a better outcome.

One prediction of Eberhard's (1985) female choice hypothesis is that exactly those male structures that are species specific should come into contact with the female. Since we are concerned with genital morphology, only physical contact between these organs is considered. The prediction is fulfilled in *N. cellulanus* as well as in some other spider species investigated (B.A. Huber, in preparation). Four structures of the *N. cellulanus* copulatory organ (paracymbium, conductor complex, median apophysis, and embolus) come into contact with the female, and it is exactly these structures that are generally used by taxonomists in species discrimination.

The critical question is whether these structures can stimulate the female during copulation, and how the female can evaluate their mechanical fit. If such stimulation was the criterion for female choice, then there should be sensory (mechanoreceptive) structures in the female contact zones. This prediction has been tested in arthropods in a very few cases (e.g., Robinson and Paterson (1982) illustrate the sense organs in the prothorax of a female damselfly, where the male grasping organs grip the female; Eberhard (1992) mentions setae on the hemisternites of female beetles that are contacted by the male's parameres), but it may be fulfilled in a wide range of animals.

In *N. cellulanus*, however, this is very probably not the case. Four types of mechanoreceptive sensilla have been found in spiders (Foelix 1985): (1) tactile hairs; (2) trichobothria; (3) slit sensilla, and (4) internal joint receptors. None of the first three types was found by scanning electron microscopy in the genital region of *N. cellulanus* females (all contact zones were scanned, as shown in Fig. 7a, with the exception of the interior of the vulval pockets). Internal joint receptors have so far only been found in association with the joint membranes of the legs (Foelix and Choms 1979; Seyfarth 1985). In the copulatory organs of *N. cellulanus* females, however, only the posterior side of the epigastric furrow is membranous; all other contact zones have a thick cuticle and are strongly sclerotized. This sheds doubt on the ability of *N. cellulanus* females to sense the various structures of the male's copulatory organ.

However, although receptors could not be found in the female's contact zones, tactile stimulation cannot be definitely excluded. There might be undiscovered internal receptors in the membranous parts of the epigastric furrow and the genital pouches. One could further argue that the structures of the male might cause stimulatory twisting or bending in regions adjacent to the female's epigyne. But theoretical considera-

tions argue against this objection: when females discriminate between males on the basis of stimulation, selection should strongly favour direct perception of the stimuli. Why do the contact zones lack sensilla whereas almost all other body surfaces are literally covered with various types of mechanoreceptors?

Another possible means for the female to evaluate the fitting of the male genitalia was indicated by Watson (1991). He stated that in *Linyphia litigiosa*, the "flub to hit ratio" is a measure of the male's competence in copulation. Such "flubs" (unsuccessful intromissions) also occur in *N. cellulanus*, but simply seem to be due to the difficulty in engaging the conductor in the small genital orifice. This may select for the male's ability to position himself appropriately, but it can hardly be a cue that allows the female to evaluate any aspect of the male's genital structures.

Another objection to the "stimulation hypothesis" concerns the assumption that the mechanical fit could be "summarized" by one single stimulus. This seems to contradict Eberhard's view (1985, p. 72) that structures in the male become more complex in order to exert additional stimuli, but it still could be that in spiders they become more complex in order to increase the strength of a single stimulus. However, this speculation is unsupported by empirical data, and might stem from the relative simplicity of explaining rapid evolutionary processes on the basis of stimuli involved.

Consequently, the question of whether Eberhard's "mechanical discrimination" is an alternative to "real" female choice (as defined above), and can explain the complexity and species specificity of spider genitalia, should be examined. About two decades ago, Kraus (1968) and Grasshoff (1974, 1975) had already expressed an idea that is probably related (Grasshoff (1975, p. 68) even mentions sexual selection in connection with genital morphology!), especially with respect to spiders. They assumed that there might be a "technischer Selektionsdruck" to ensure sperm transfer. This could constantly force males to evolve additional and increasingly complex structures to ensure sperm transfer in spite of morphological variance in the genitalia of females (= varying female "preferences"). This process could begin the evolutionary diversification of species as soon as they have become separated by any isolating mechanism, and it might be continued independently to an extreme point, owing to the high degree of evolutionary freedom in genitalia compared with other organ systems (Grasshoff 1975). In this case, the selective force that favours or discriminates against a certain variant of the male copulatory organs is not exerted by any female behavioural or physiological response to the male trait, but indirectly by influencing the number of offspring that are assumed to result from the sperm that are transferred to the female spermathecae. This assumption, that the fitting of the genitalia is closely correlated with the number of sperm transferred, was not tested in *N. cellulanus*.

It is not a strict prerequisite of the hypothesis outlined that females must have genitalic contact with more than one male (as assumed by Eberhard 1985). This is in accordance with the present finding that *N. cellulanus* females generally do not accept a second male, although more evidence for this is needed ( $N = 5$  only; all observations were performed on animals in captivity).

In conclusion, the "technische Selektion" hypothesis of Kraus (1968) and Grasshoff (1975) and the female choice

hypothesis of Eberhard (1985) (including "mechanical discrimination") seem to be complementary, and together may provide a convincing explanation for species specificity and complexity in animal genitalia.

### Acknowledgements

I am grateful to G. Pass for laboratory facilities, for reading the manuscript, and for helpful criticism. Special thanks are extended to A. Tadler and K. Thaler for their help and encouragement as well as for many valuable discussions during this study. The help of M. Landolfi and two anonymous reviewers, who provided valuable comments and linguistic suggestions, is gratefully acknowledged.

- Bhatnagar, R.D.S., and Rempel, J.G. 1962. The structure, function, and postembryonic development of the male and female copulatory organs of the black widow spider *Latrodectus curacaviensis* (Müller). *Can. J. Zool.* 40: 465–510.
- Bhatnagar, R.D.S., Singh, G., and Sadana, G.L. 1971. Structure and function of the male and female external genitalia of *Clubiona drassodes* Cambridge (Clubionidae; Araneae). *Zool. Pol.* 21: 29–38.
- Coddington, J.A. 1990. Ontogeny and homology in the male palpus of orb-weaving spiders and their relatives, with comments on phylogeny (Araneoclad: Araneoidea, Deinopoidea). *Smithson. Contrib. Zool.* No. 496. pp. 1–52.
- Cooke, J.A.L. 1966. Synopsis of the structure and function of the genitalia of *Dysdera crocata* (Araneae, Dysderidae). *Senckenb. Biol.* 47: 35–43.
- Costa, F.G., and Sotelo, J.R. 1984. Influence of temperature on the copulation duration of *Lycosa malitiosa* Tullgren (Araneae, Lycosidae). *J. Arachnol.* 12: 273–277.
- Darwin, C. 1871. The descent of man and selection in relation to sex. (Reprinted.) Modern Library, New York.
- Dumitrescu, M. 1979. La monographie des représentants du genre *Nesticus* des grottes de Roumanie. I<sup>re</sup> Note. *Trav. Inst. Speol. Emile Racovitza*, 18: 53–84.
- Dumitrescu, M. 1980. La monographie des représentants du genre *Nesticus* des grottes de Roumanie II<sup>e</sup> Note. *Trav. Inst. Speol. Emile Racovitza*, 19: 77–101.
- Eberhard, W.G. 1985. Sexual selection and animal genitalia. Harvard University Press, Cambridge.
- Eberhard, W.G. 1992. Species isolation, genital mechanics, and the evolution of species-specific genitalia in three species of *Macro-dactylus* beetles (Coleoptera, Scarabaeidae, Melolonthinae). *Evolution*, 46(6): 1774–1783.
- Engelhardt, V. 1910. Beiträge zur Kenntnis der weiblichen Copulationsorgane einiger Spinnen. *Z. Wiss. Zool.* 96: 32–117.
- Foelix, R.F. 1985. Mechano- and chemoreceptive sensilla. In *Neurobiology of arachnids*. Edited by F.G. Barth. Springer-Verlag, Berlin, Heidelberg, and New York. pp. 118–137.
- Foelix, R.F., and Choms, A. 1979. Fine structure of a spider joint receptor and associated synapses. *Eur. J. Cell Biol.* 19: 149–159.
- Gerhardt, U. 1927. Neue biologische Untersuchungen an einheimischen und ausländischen Spinnen. *Z. Morphol. Oekol. Tiere*, 8: 96–186.
- Gering, R.L. 1953. Structure and function of the genitalia in some American agelenid spiders. *Smithson. Misc. Collect.* 121: 1–84.
- Gertsch, W.J. 1984. The spider family Nesticidae (Araneae) in North America, Central America and the West Indies. *Tex. Mem. Mus. Bull.* 31: 1–91.
- Grasshoff, M. 1968. Morphologische Kriterien als Ausdruck von Artgrenzen bei Radnetzspinnen der Subfamilie Araneinae (Arachnida: Araneae: Araneidae). *Abh. Senckenb. Naturforsch. Ges.* 516: 1–100.
- Grasshoff, B. 1973. Bau und Mechanik der Kopulationsorgane der

- Radnetzspinne *Mangora acalypha* (Arachnida, Araneae). Z. Morphol. Tiere, 74: 241–252.
- Grasshoff, M. 1974. Zur Evolution der Kopulationsorgane der Spinnen. Nat. Mus. 104: 55–60.
- Grasshoff, M. 1975. Die Evolution komplizierter Kopulationsorgane—ein separater Adaptationsverlauf. Aufs. Red. Senckenb. Naturforsch. Ges. 27: 61–68.
- Heimer, S. 1982. Interne Arretierungsmechanismen an den Kopulationsorganen männlicher Spinnen (Arachnida, Araneae). Entomol. Abh. (Dresden), 45: 35–64.
- Jackson, R.R. 1980. The mating strategy of *Phidippus johnsoni* (Araneae, Salticidae): II. Sperm competition and the function of copulation. J. Arachnol. 8: 217–240.
- Kraus, O. 1968. Isolationsmechanismen und Genitalstrukturen bei wirbellosen Tieren. Zool. Anz. 81(1/2): 22–38.
- Lehtinen, P.T., and Saaristo, M.I. 1980. Spiders of the Oriental–Australian region. II. Nesticidae. Ann. Zool. Fenn. 17: 47–66.
- Loebroks, A. 1983. Revision der Krabbenspinnen-Gattung *Heriades* Simon (Arachnida: Araneae: Thomisidae). Verh. Naturwiss. Ver. Hambg. 26: 85–139.
- Loebroks, A. 1984. Mechanik der Kopulationsorgane von *Misumenavatia* (Clerck, 1757) (Arachnida: Araneae: Thomisidae). Verh. Naturwiss. Ver. Hambg. 27: 383–403.
- Lopez, A. 1987. Glandular aspects of sexual biology. In Ecophysiology of spiders. Edited by W. Nentwig. Springer-Verlag, Berlin, Heidelberg, and New York. pp. 121–132.
- Martin, D. 1981. Bau und Funktion der Kopulationsorgane bei Zergspinnen: 1. *Tapinocyba insecta* (L. Koch) (Arachnida, Araneae, Erigonidae). Entomol. Abh. (Dresden), 44: 81–86.
- Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge.
- Melchers, M. 1963. Zur Biologie und zum Verhalten von *Cupiennius salei* (Keyserling), einer amerikanischen Ctenide. Zool. Jahrb. Abt. Syst. Oekol. 91: 1–90.
- Osterloh, A. 1922. Beiträge zur Kenntnis des Kopulationsapparates einiger Spinnen. Z. Wiss. Zool. 119: 326–421.
- Robinson, H.M., and Patterson, H.E.H. 1982. Mate recognition and mechanical isolation in *Enallagma* damselflies (Odonata: Coenagrionidae). Evolution, 36: 243–250.
- Romeis, B. 1989. Mikroskopische Techniken. 17th ed. Urban & Schwarzenberg, Vienna.
- Rovner, J.S. 1972. Copulation in the lycosid spider *Lycosa rabida* (Walckenaer): a quantitative study. Anim. Behav. 20: 133–138.
- Rovner, J.S. 1973. Copulatory pattern supports generic placement of *Schizocosa avida* (Walckenaer) (Araneae: Lycosidae). Psyche (Camb.), 80: 245–248.
- Rovner, J.S. 1974. Copulation in the lycosid spider *Schizocosa saltatrix* (Hentz): an analysis of palpal insertion patterns. Anim. Behav. 22: 94–99.
- Sadana, G.L. 1972. Mechanics of copulation in *Lycosa chaperti* Simon (Araneida: Lycosidae). Bull. Br. Arachnol. Soc. 2: 87–89.
- Schult, J., and Sellenschlo, U. 1983. Morphologie und Funktion der Genitalstrukturen bei *Nephila* (Arach., Aran., Araneidae). Mitt. Hamb. Zool. Mus. Inst. 80: 221–230.
- Seyfarth, E.A. 1985. Spider proprioception: receptors, reflexes, and control of locomotion. In Neurobiology of arachnids. Edited by F.G. Barth. Springer-Verlag, Berlin, Heidelberg, and New York. pp. 230–248.
- Shapiro, A.M., and Porter, A.H. 1989. The lock-and-key hypothesis: evolutionary and biosystematic interpretation of insect genitalia. Annu. Rev. Entomol. 34: 231–245.
- Shear, W.A. 1967. Expanding the palpi of male spiders. Breviora, 259: 1–27.
- Starr, C.K. 1988. Sexual behavior in *Dictyna volucris* (Araneae, Dictynidae). J. Arachnol. 16: 321–330.
- Tretzel, E. 1954. Reife- und Fortpflanzungszeit bei Spinnen. Z. Morphol. Oekol. Tiere, 42: 634–691.
- Uetz, G.W., and Denterlein, G. 1979. Courtship behavior, habitat, and reproductive isolation in *Schizocosa roseni* Uetz and Dondale (Araneae: Lycosidae). J. Arachnol. 7: 121–128.
- van Helsdingen, P.J. 1965. Sexual behaviour of *Lepthyphantes leprosus* (OHLERT) (Araneida, Linyphiidae), with notes on the function of the genital organs. Zool. Meded. (Leiden), 41(2): 15–42.
- van Helsdingen, P.J. 1969. A reclassification of the species of *Linyphia* Latreille based on the functioning of the genitalia (Araneida, Linyphiidae). Part I. *Linyphia* Latreille and *Neriene* Blackwell. Zool. Verh. (Leiden), 105: 1–303.
- Watson, P.J. 1991. Multiple paternity as genetic bet-hedging in the female sierra dome spiders, *Linyphia litigiosa* (Linyphiidae). Anim. Behav. 41: 343–360.
- Weiss, I. 1979. Das Männchen von *Phlegma m-nigra* (Kulczynski, 1891), nebst Betrachtungen über Bau und Funktion der Kopulationsorgane mitteleuropäischer Arten der Gattung *Phlegma* s.l. (Arachnida: Araneae: Salticidae). Stud. Com. Mus. Brukenthal, 23: 239–250.
- Weiss, I. 1981. Der Kopulationsmechanismus bei *Nesticus cibiniensis* n.sp., einer neuen Höhlenspinne aus Rumänien (Arachnida, Araneae, Nesticidae). Reichenbachia, 19: 143–152.
- Weiss, I. 1982. Konstruktions- und Funktionsanalyse der Kopulationsorgane von *Zodariion aurora* n.sp. aus Rumänien (Arachnida, Araneae, Zodariidae). Reichenbachia, 20: 77–83.
- Weiss, I. 1983. *Carpathonesticus lotriensis* n.sp., eine neue Höhlenspinne aus Rumänien (Arachnida, Araneae, Nesticidae). Reichenbachia, 21: 107–112.
- Weiss, I. 1989. Über *Oxyopes nigripalpis* Kulcz. und *O. lineatus* Latr. (Arachnida, Araneae, Oxyopidae). Reichenbachia, 27: 1–4.
- Weiss, I., and Heimer, S. 1982. Zwei neue *Carpathonesticus*-Arten aus Rumänien nebst Betrachtungen über Kopulationsmechanismen und deren Evolution (Arachnida, Araneae, Nesticidae). Reichenbachia, 20: 167–174.