Sexual morphology of male *Sepsis cynipsea* (Diptera: Sepsidae): lack of support for lock-and-key and sexually antagonistic morphological coevolution hypotheses

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Abstract—Traits that function in male–female sexual interactions tend to diverge rapidly compared with other traits. Several hypotheses attempt to explain this evolutionary pattern. Predictions of two of these hypotheses, lock-and-key and sexually antagonistic morphological coevolution, were tested by examining how two sets of species-specific male structures fit with female structures during courtship and copulation in the fly *Sepsis cynipsea* (L., 1758). Contrary to predictions of both hypotheses, neither the species-specific modifications of the male's front legs nor those of his genitalic surstyli were matched by modifications of the female structures with which they meshed (wing bases, 6th abdominal sternite); males damaged small patches of microtrichia on the female's wings with their legs, but the morphology of the female's wings and abdomen showed no sign of the defensive designs expected under antagonistic morphological coevolution. Data regarding the alternative hypothesis of sexually antagonistic behavioral coevolution by females in response to male morphology were less conclusive, but this hypothesis failed to explain the sustained, apparently stimulatory rhythmic squeezing by the male genitalia and the lack of female defensive responses to this squeezing. These movements of the male surstyli during copulation suggest that they function to stimulate the female. The wing base of the female has apparent sense organs near the sites contacted by the male, as expected under the alternative hypothesis of traditional female choice to explain rapid divergent evolution. The male's genitalic surstyli were also used in novel precopulatory interactions. A pair of previously undescribed processes at the bases of the surstyli probably grasp and may also rhythmically squeeze the female during copulation.

Résumé—Les caractères impliqués dans les interactions sexuelles entre les mâles et les femelles ont tendance à diverger plus rapidement que les autres caractères. Plusieurs hypothèses cherchent à expliquer ce patron évolutif. L'examen de l'appariement de deux appareils mâles spécifiques aux structures femelles durant la cour et l'accouplement chez la mouche *Sepsis cynipsea* (L., 1758) a permis de vérifier les prédictions de deux de ces hypothèses, celle de la serrure et de la clé et celle de la coévolution morphologique sexuelle antagoniste. Contrairement aux prédictions des deux hypothèses, les femelles ne possèdent pas de modifications des structures (bases des ailes, 6e sternite abdominal) qui sont en interaction avec les modifications spécifiques des pattes antérieures des mâles, ni avec celles de leurs surstyles génitaux. Les mâles endommagent de petites plages de microtriches sur les ailes des femelles avec leurs pattes; la morphologie de la femelle ne montre, cependant, aucune signe des adaptations de l'aile et de l'abdomen prévues par la coévolution morphologique antagoniste. Les données concernant l'hypothèse de rechange de la coévolution du comportement sexuel antagoniste chez la femelle en réaction à la morphologie du mâle sont encore moins claires; l'hypothèse ne réussit pas à expliquer la compression rythmique soutenue, apparemment stimulatrice, exercée par les génitalias mâles et l'absence de réactions de défense de la part de la femelle à cette compression. Les mouvements des surstyles mâles durant l'accouplement laissent croire qu'ils servent à stimuler la femelle. La base de l'aile de la femelle possède, semble-t-il, des organes sensoriels près des surfaces palpées par le mâle, tel que prévu par l'hypothèse de rechange traditionnelle du choix de la femelle pour expliquer l'évolution divergente rapide. Les surstyles génitaux du mâle servent aussi à des interactions précopulatrices inédites : une paire de processus, encore non décrits, à la

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base retiennent probablement la femelle et la compriment aussi sans doute rythmiquement durant la copulation.

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Introduction

The functional morphology of both the front legs and the genitalic surstyli of male sepsid flies (Diptera) is of general interest for a number of reasons. Both structures have diverged rapidly in many groups, and they often have species-specific forms even among closely related species (Duda 1925, 1926; Pont 1979; Steyskal 1987; Ozerov 1992, 1993; Silva 1993), as is typical of male genitalia in general (Eberhard 1985). In addition, their mechanical functions are apparently simple and clear throughout the family: the male's front legs clasp the female's wings after he mounts her (Šulc 1928; Pont 1979; Eberhard 2001*a*), and his genitalic surstyli grasp the external surface of her abdomen during copulation (Eberhard and Pereira 1996; Eberhard 2001*b*, 2001*c*). Sepsid front legs and surstyli can thus be used to test general hypotheses that attempt to explain the common evolutionary trend for male structures that are specialized to contact females to frequently show rapid divergent evolution (Eberhard 1985).

Two sets of hypotheses that attempt to explain such sustained rapid divergent evolution make contrasting predictions that can be applied to sepsids. One set, which includes the lock-and-key hypothesis (Shapiro and Porter 1989) and the antagonistic coevolution hypothesis (Alexander *et al*. 1997; Holland and Rice 1998; Chapman *et al.* 2003), predicts that male and female morphology will tend to coevolve. Species-specific male traits should fit with or otherwise be adjusted to species-specific differences in females. Thus the species-specific aspects of male front leg and surstylus morphology should fit with species-specific female wing and abdomen morphology in ways that enhance the male's ability to hold on to the female. The sexually antagonistic morphological coevolution hypothesis also predicts that the species-specific aspects of the designs of coevolving female structures should often be appropriate to defend against species-specific male traits (Eberhard 2004). Female wing and abdomen morphology should have species-specific properties that can impede the grasp of conspecific or heterospecific males.

Sexual selection by female choice, in contrast, predicts that male–female morphological coevolution will often (though not always) fail to occur. Lack of coevolution is especially likely when female selectivity results from differences in her sensitivity to stimulation from the male (presumably due to differences in the structure and properties of her sense organs or her nervous system), rather than from differences in mechanical fit. A second prediction of female choice is that, because the male legs and genitalia would function to stimulate the female by touch, there should be sensory receptors at or near the sites that are contacted by males. Female morphological designs to defend against the grasping action of male structures could be present, but they are not necessary predictions of female choice.

Behavioral versions of the sexually antagonistic coevolution hypothesis are also compatible with a lack of species-specific female morphology. Females should behave in ways that impair the function of the male structures with species-specific morphology (*i.e.*, impede his grasping of her wings and abdomen), and this behavior should vary among species. The differences among females of different species should be in some sense complementary to male morphology; the male's morphology should be designed to allow him to hold the female despite her species-specific resistance behavior.

Following the pioneering studies of Parker (1972*a*, 1972*b*), the sepsid fly *Sepsis cynipsea* (L., 1758) has become a model species for studies of sexual selection. These studies include measurements of selection intensity under different conditions (Blanckenhorn 1999), strategic ejaculation (Martin and Hosken 2002), mating costs (Martin *et al.* 2003), fluctuating asymmetry (Allen and Simmons 1996), sexual conflict (Blanckenhorn *et al.* 2002, Martin and Hosken 2003), female choice and resistance behavior (Blanckenhorn *et al.* 2000; Ding and Blanckenhorn 2002), and direct male–male competition (Ward 1983; Ward *et al.* 1992). Nevertheless, knowledge of how sexually dimorphic morphological traits function in this species, including how the male's modified front legs clamp the female's wings and how

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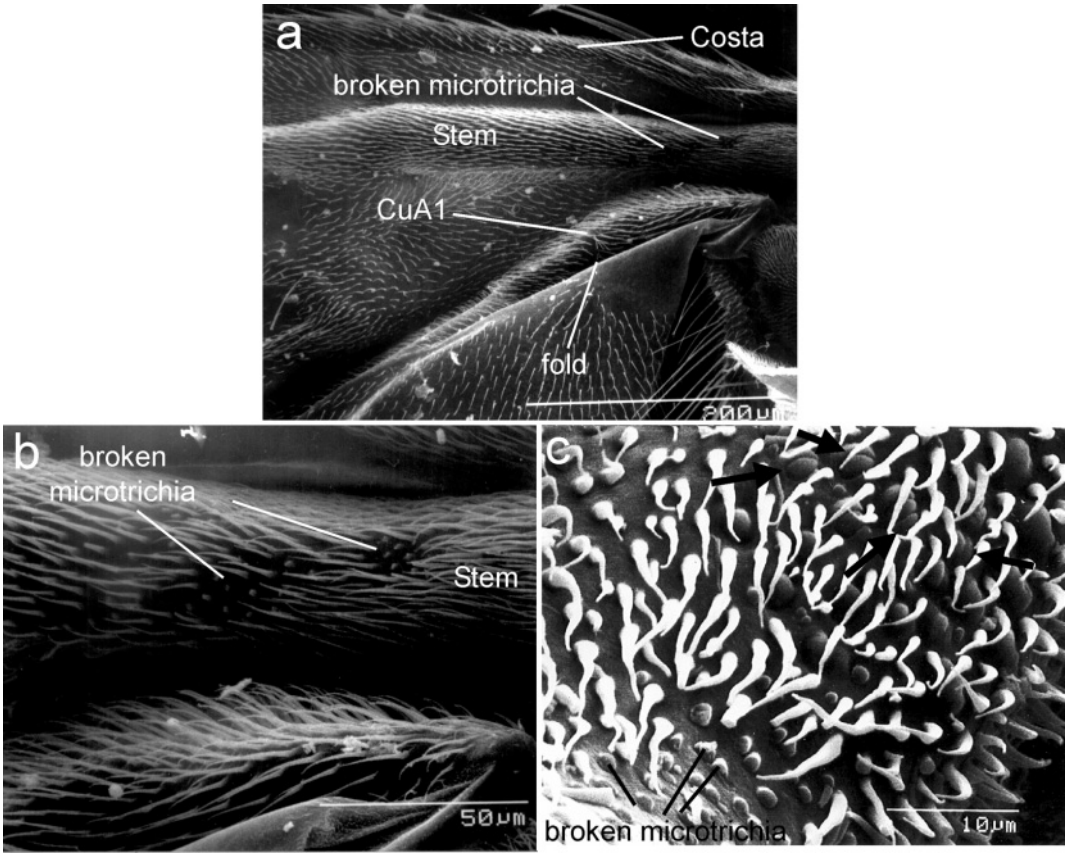
Fig. 1. Wings of female *Sepsis cynipsea*. (*a*) Dorsal overview of the base of the wing; (*b*) patches of broken microtrichia on the Stem vein where the male's front femur grasped the wing; (*c*) view of same area of a different wing from angle more or less parallel to intact microtrichia, revealing the campaniform sensilla (black arrows) near but not in the area contacted by the male.



his genitalic surstyli engage her abdomen, has lagged behind that for sepsids in the genera *Archisepsis*, *Palaeosepsis*, and *Microsepsis* (Eberhard and Pereira 1996; Eberhard 2001*a*, 2001*b*, 2002*a*, 2002*b*, 2003) (these genera form a single clade, sometimes called *Palaeosepsis*; see Meier 1993). This paper describes several aspects of the sexual behavior and functional morphology of these sexually dimorphic structures in *S. cynipsea*. The large genus *Sepsis*, which contains nearly half of all sepsid species (Meier 1993), has traditionally been classified in the same subfamily (Sepsinae) as *Palaeosepsis* s.l. (Duda 1925). A recent cladistic analysis was in general accord, although it differed in some details (Meier 1993).

Materials and methods

Fly behavior was observed and videotaped using a digital camera with +7 closeup lenses in cow pastures in Fehraltsdorf, near Zurich, Switzerland, and in captivity through a dissecting microscope. Copulating pairs were frozen in the field with ethyl chloride spray and fixed immediately in 80% ethanol; solitary individuals were collected directly into 80% ethanol. For examination with a scanning electron microscope (SEM), specimens were fixed in Karnovsky's fixative (glutaraldehyde–paraformaldehyde), dehydrated, dried by sublimation, and sputter-coated with 20-nm gold particles. Female wings and male hypandria from specimens in ethanol were mounted on microscope slides in euparol to study campaniform sense organs (wings) and muscles (hypandria).

Results

Front-leg clamp on female wing

The male grasped the bases of the female's

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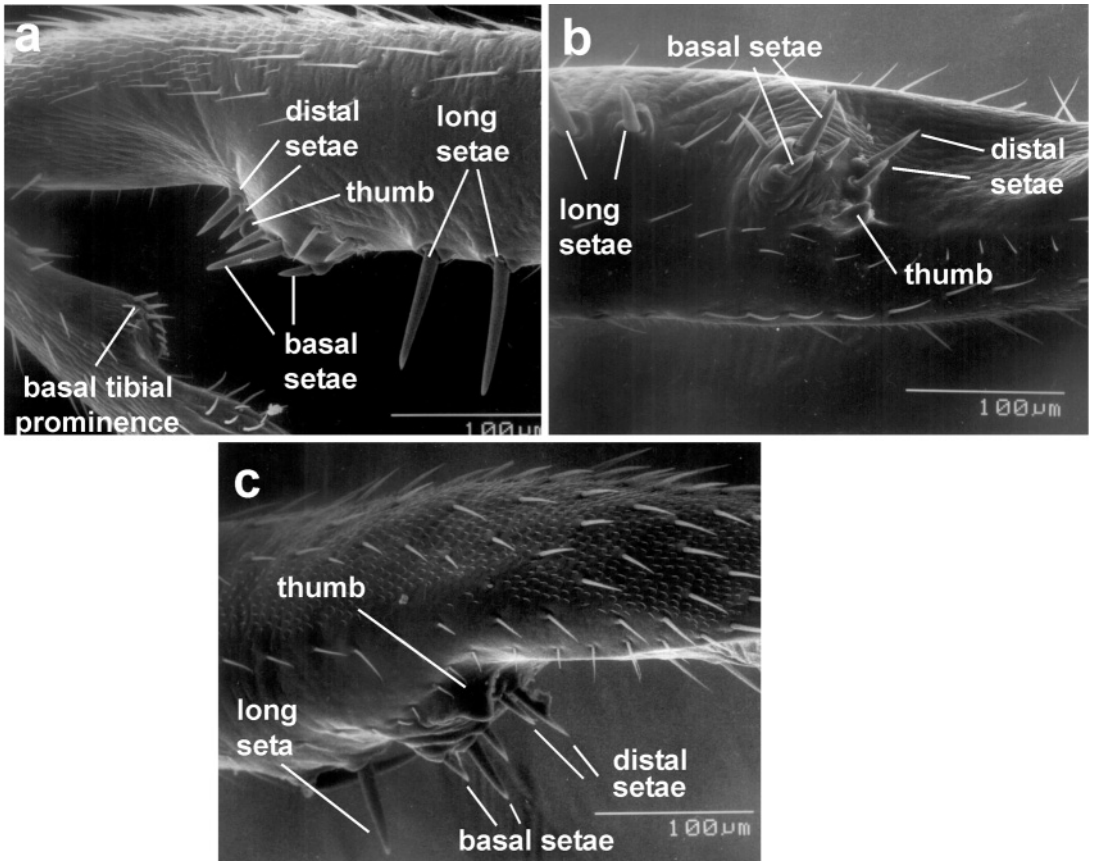
Fig. 2. Front legs of male *Sepsis cynipsea* in anterior (*a*), ventral (*b*) and posterior (*c*) views.



wings with his front legs before and during copulation. The veins on the dorsal surfaces of the female's wings that were most important for the male's grip were the Costa vein at the anterior edge, the thick Stem vein just posterior to the Costa vein, and the CuA1 vein, which had a deep fold along its posterior border (Fig. 1*a*). The Stem vein bore several apparent campaniform sense organs in this area (three are indicated with arrows in Fig. 1*c*), which was grasped by the male's femur (below). Wings of females that had been mounted by males had small patches of broken microtrichia on the posterior-dorsal surface of the Stem vein (Figs. 1*b*, 1*c*) where it had been contacted by the male's femur.

The male's front femoral clasping structure, which clamped the dorsal surface of the female's wing, had a distal group of five setae, a small flattened plate or "thumb" with a curved ventral edge, and two long basal setae (Figs. 2*a*–2*c*). When the front leg grasped the female's wing (Fig. 3), four setae in the distal group bracketed the Stem vein. The two most distal setae pressed on the anterior surface of the Stem vein (Figs. 3*b*, 3*c*), and the two most basal setae on its posterior surface (Fig. 3*c*). The curved ventral margin of the thumb pressed against the dorsal surface of the Stem vein (Fig. 3*b*).The small medial seta of the distal group pressed against the dorsal surface of the Stem vein and was deflected sharply posteriorly (Fig. 3*c*). More posteriorly, the two large basal setae lodged in the deep fold just posterior to the CuA1 vein (Fig. 3*a*), where their tips converged on each other (Fig. 3*d*). When examined under the light microscope, this fold in the female's wing showed no obvious modifications such as thickening in the area that was contacted by these setae.

The male's front tibia pressed against the ventral surface of the female's wing. Here the important landmarks for the male's grasp were the Costa vein, the basicostal cell, and the br cell (Fig. 4*a*). There was a cluster of three campaniform sensilla (Fig. 4*b*) approximately

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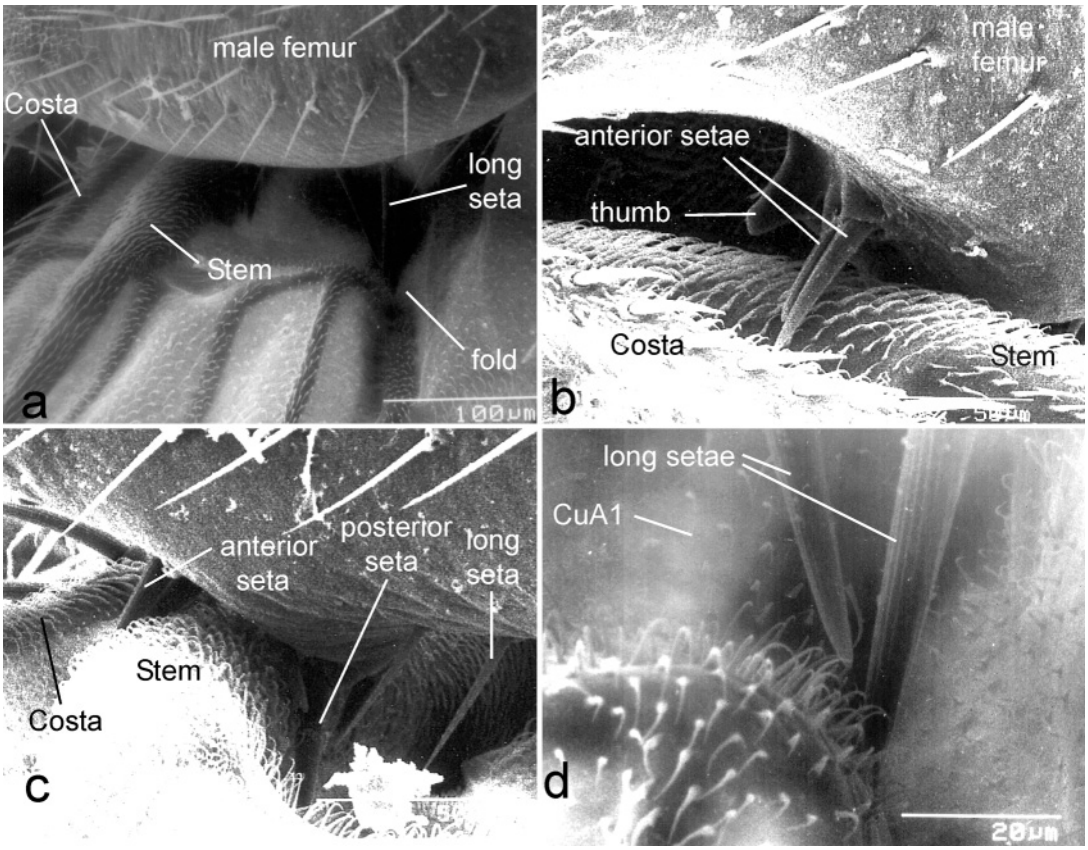
Fig. 3. Front femur of male *Sepsis cynipsea* clamps the dorsal surface of the female's wing. (*a*) Dorsolateral view; (*b*) close-up anterolateral view, showing how the anterior pair of distal femoral setae press on the anterior surface of the Stem vein and the curved ventral surface of the male's plate ("thumb") presses on the dorsal surface of the Stem vein; (*c*) close-up posterolateral view, showing how the posterior pair of femoral setae press on the posterior surface of the Stem vein and the long basal setae of the femur are inserted into the fold in the wing just posterior to the CuA1 vein; (*d*) close-up of the tips of the two long basal setae of the femur converging in the wing fold just posterior to the CuA1 vein.



50 µm basal to the area grasped by the male's tibia. The basal prominence of the male's front tibia fit into the basicostal cell, while the distal prominence of his tibia pressed on the br cell (Fig. 4*c*).

Genitalic mesh prior to copulation

Males in the field and in captivity used their surstyli in two precopulatory behavioral patterns that have not been described in other sepsids. In the field the tips of the male's surstyli, which bore short, stout setae (Fig. 5*b*), repeatedly engaged the dorsal surface of the female's abdomen (probably between tergites 6 and 7) for periods of up to 30 s. These were probably the "temporary genital contacts" that Parker (1972*a*) described as sometimes lasting up to 2–3 min. In five cases in which my angle of observation was favorable, the male initiated genital contact of this sort by directing his surstyli ventrally about 90° away from their resting position near the ventral surface of his abdomen (Fig. 5*a*) and "striking" (or stroking: Parker 1972*a*) rapidly at the dorsal surface of the tip of the female's abdomen. Strikes were generally preceded by short bursts of rapid abdominal quivering.

During temporary genitalic contacts the female generally extended her ovipositor posteriorly one or more times beyond the male's genitalia. These extensions indicate that the tips of the male's genitalic surstyli must have been dorsal to her vulva, as otherwise his surstyli would have prevented such extensions. The male's intromittent genitalia, which are near the bases of his surstyli, were thus relatively distant

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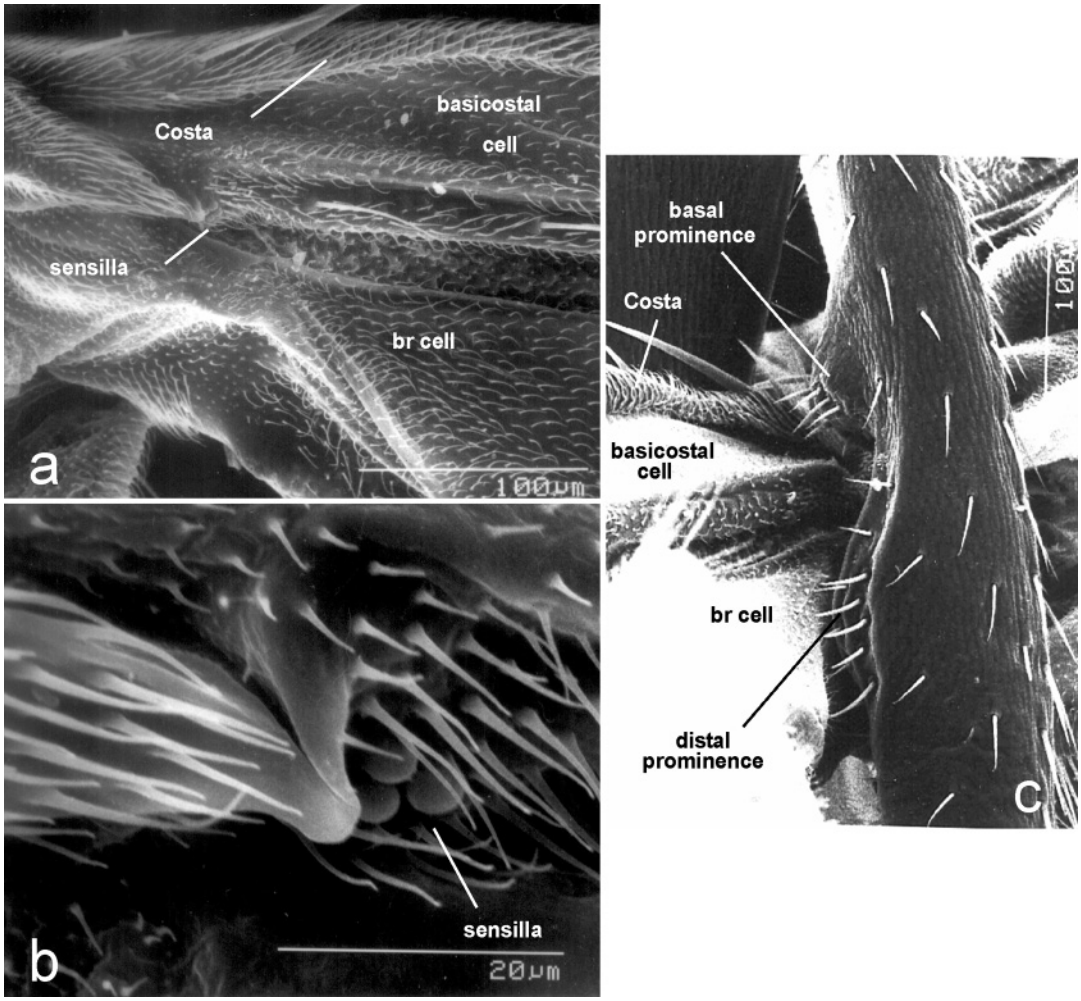
Fig. 4. Ventral view of the female's wing and the clamp by the male's front tibia (the male's clamp has opened slightly). (*a*) Ventral surface of wing base of female; (*b*) close-up of group of sensilla on the Stem vein; (*c*) basal and distal tibial prominences of the male mesh with the basicostal and br cells of the wing.



from the female's vulva during temporary genitalic contact; these sustained genitalic contacts were thus probably not intromission attempts. The male generally pulled the female's abdomen dorsally while contacting her with the tips of his surstyli, and her abdomen often immediately descended as soon as contact ended. Temporary genitalic engagements in the field often ended with a sharp ventral "snap" of the female's abdomen (or perhaps a dorsal snap of the male's abdomen?). Some temporary engagements ended when the female pushed the male's genitalia away with the tips of her hind tibiae.

Precopulatory genitalic contacts of the male's surstyli with the female's abdomen also occurred in captivity but differed in that they were usually longer (often several minutes), and the male's abdomen was positioned more posteriorly with respect to that of the female. The tip of his abdomen curved ventrally and covered both the female's proctiger and the tip of her ovipositor, and the tips of his surstyli were near the posterior edge of the female's 6th sternite. These may have been intromission attempts, as the male's intromittent genitalia were close to the female's vulva. It was not clear, however, whether the female's proctiger was raised, which must occur if penetration is to occur (Eberhard 2002*c*).

Genitalic mesh during copulation

During copulation, the long male surstyli

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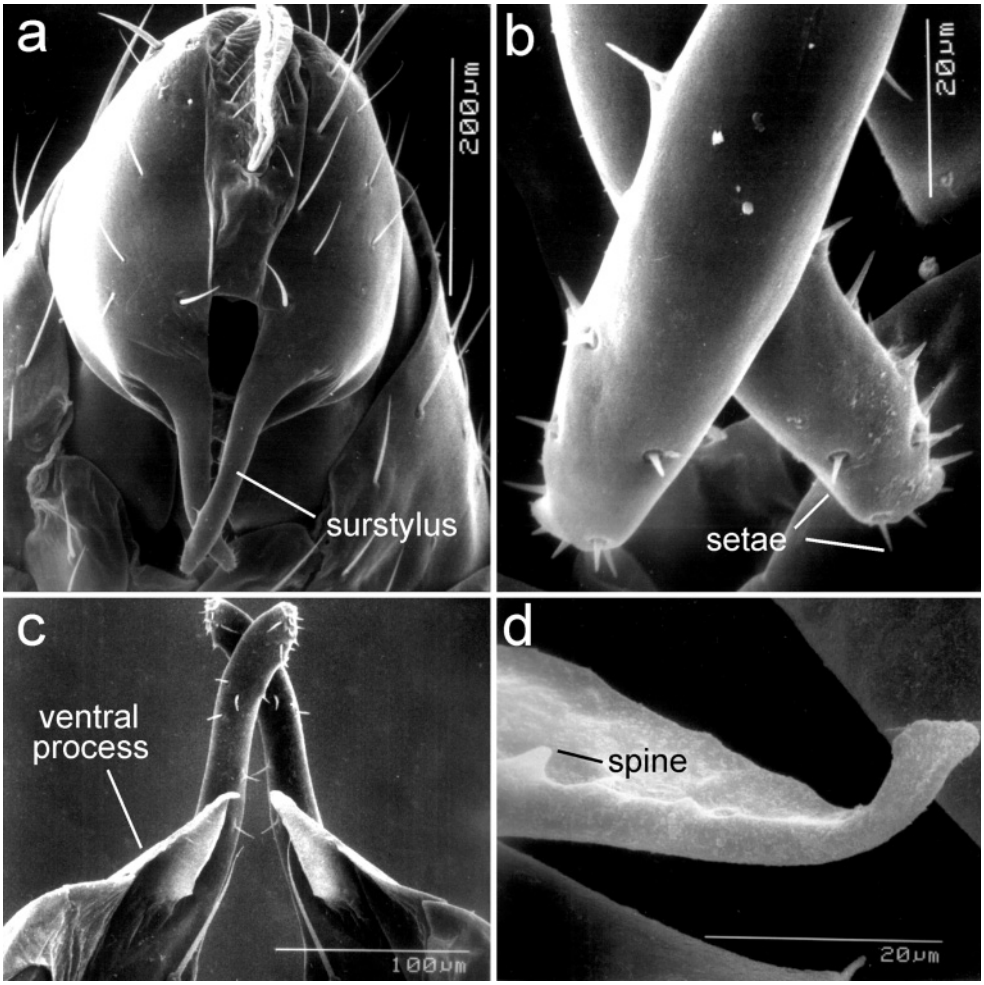
Fig. 5. Genitalic surstyli of a male *Sepsis cynipsea* (intromittent genitalic structures not visible). (*a*) Resting position (dorsal view), folded against the male's ventral surface; (*b*) close-up dorsal view of the setae at the tips of the surstyli; (*c*) ventral view of surstyli, showing the ventral processes at their bases; (*d*) approximately lateral close-up view of one ventral process, showing teeth and hook at tip.



grasped the female's pleural membranes tightly at the sides of her 6th sternite, and in some pairs the tips of the surstyli caused these membranes and the lateral margins of the sternite to fold sharply inward (Figs. 6, 7). The small ventral process at the base of each surstylus (Figs. 6–8) also appeared to contact the female's pleural membranes (Figs. 6*c*, 7*a*). The tips of the small ventral processes were relatively transparent under the light microscope (Fig. 8*a*), suggesting that they are relatively soft. The presence of muscles inside the male hypandrium suggested that the small ventral processes could be moved independently (Fig. 8*b*). The large fan of muscle fibers that was attached at one end to a central sclerite between the male's two epandria (Fig. 8*b*) had two subunits. One was attached to the inner surface of the lateral wall and was apparently designed to close the entire epandrium medially, while the other was attached to the wall of the small ventral process and was apparently designed to move this process dorsomedially (Fig. 8*b*).

Observations of living flies under a dissecting microscope showed that the male surstyli squeezed the female rhythmically for periods of up to at least 2 min. Initiation of copulation was not observed in captivity, so the timing of squeezing during the approximately 22-min copulation (Parker 1972*a*) was not determined. The lateral edges of the female's 6th abdominal

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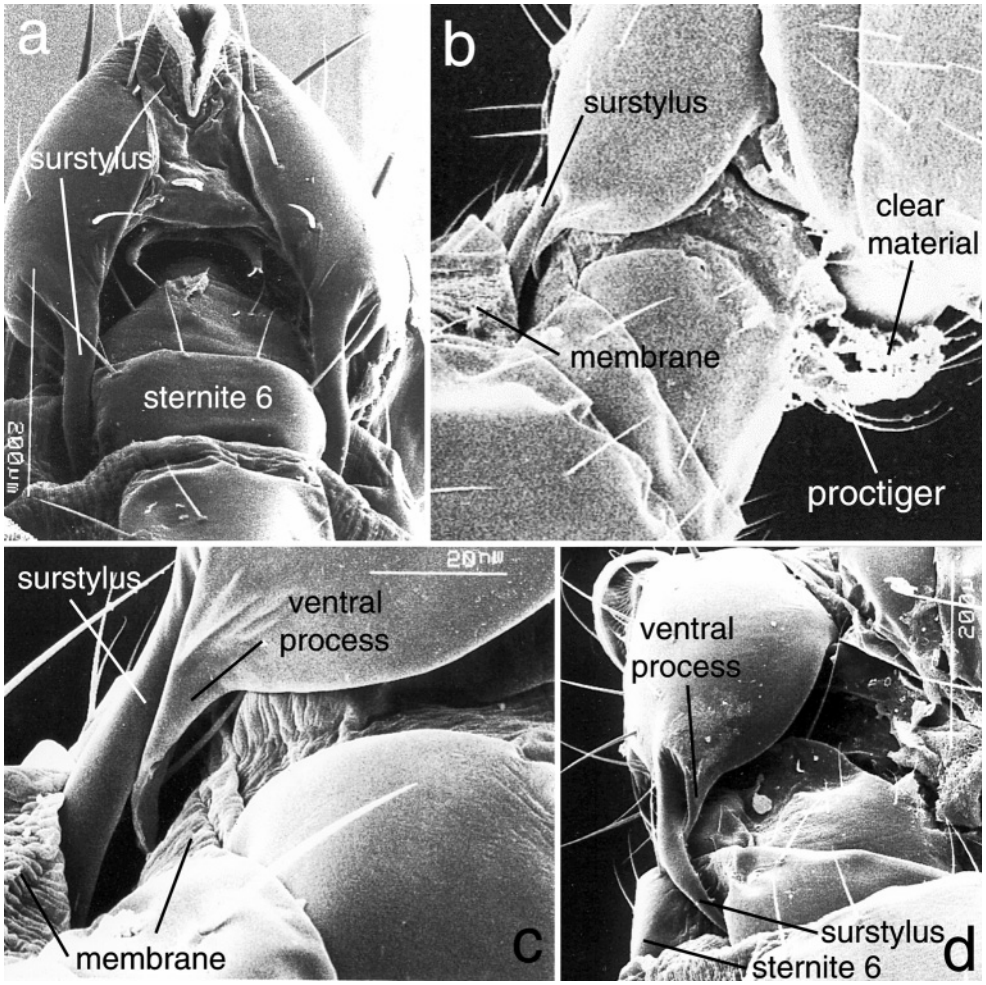
Fig. 6. Mesh of genitalia of male and female *Sepsis cynipsea* during copulation. (*a*) Ventral view of female sternites 5, 6, and 7 with male surstyli grasping the sides of sternite 6; (*b*) lateral view, showing the dorsally flexed female proctiger, the accumulated material nearby, and the surstylus deeply embedded in the female's pleural membranes lateral to her 6th sternite; (*c*) close-up lateral view of male surstylus and its ventral process grasping the female's membrane; (*d*) lateral view of male surstylus bending female sternite 6 substantially while grasping it.



sternite curled dorsally into her body each time the male squeezed (Figs. 6*a*, 6*d*); the most powerful squeezes bent each edge of the sternite approximately 90°. The tip of each surstylus was sometimes inserted deep into the fold of the intersegmental membrane (Figs. 6*c*, 7) and lay near the small seta near the anterolateral corner of the female's 6th sternite (inset in Fig. 9*d*). Inspection under the SEM revealed no campaniform sensilla on this sternite. A second type of surstylus movement was a quick, low-amplitude medial "snap", which may have involved movements of the small ventral processes (I could not see these processes directly, but observed deflections of female membranes). In some pairs the male surstyli were immobile for 2–4 min as they squeezed the sternite tightly.

In two pairs observed under the dissecting microscope, the tips of the male's surstyli seemed to rest free on the surface of the female's sternite shortly before the end of copulation, and in one frozen copulating pair the tips of the male's surstyli also did not grip the

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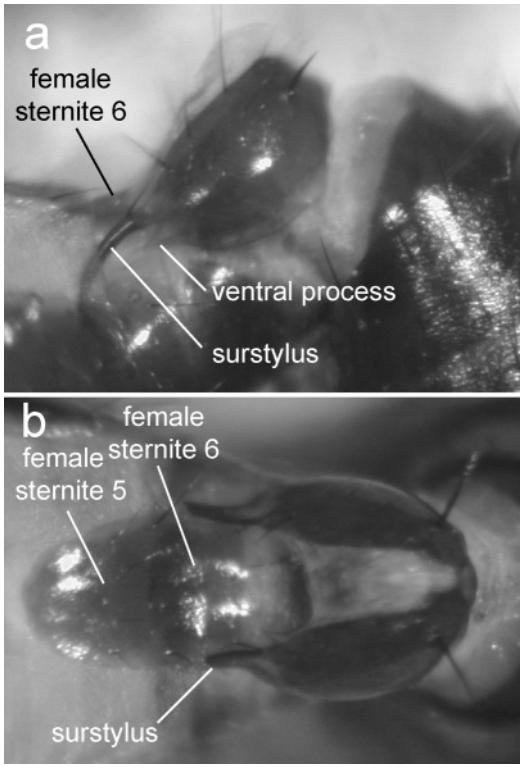
Fig. 7. Genitalic grasp of male *Sepsis cynipsea* under a dissecting microscope. (*a*) Lateral view of male genitalic surstylus extending far into the fold of the intersegmental membrane of the female, and its ventral process also making contact with the female's membrane; (*b*) ventral view of female sternites 5 and 6, with sternite 6 (and the male's surstyli) partially telescoped under sternite 5.



female (Fig. 9*c*). In still another living pair, the tips of the surstyli rested on the surface of the female's 6th sternite and then moved anteriorly and buried themselves in folds of the intersegmental membrane just anterior to this sternite for about 3 min, just before the male dismounted to end copulation.

In some pairs preserved in alcohol there was a small mass of clear, white material near the base of the male's genitalia, on or near the female's proctiger (Fig. 6*b*), similar to that seen in some other species (Eberhard 2001*c*, 2003). The female's proctiger was flexed dorsally during copulation (Fig. 6*b*), as in other sepsids (Eberhard 2002*c*); it had apparent sensilla (Figs. 9*a*, 9*b*), which could be chemosensors.

In most pairs the female rubbed her hind legs together in apparent cleaning movements during copulation. These movements often included repeated forceful contact with the male's genitalia. In two cases I saw that the male pressed the tip of his abdomen anteriorly each time the female began to rub her legs, in apparent attempts to "hide" his genitalia by burying them partially in her soft abdominal membranes. There was no sign, however, that female rubbing forced the male to end copulation, as termination was not obviously associated with female rubbing. Termination was initiated by the male dismounting, and he sometimes turned in a circle one or more times before coming free (Parker 1972*a*).

Discussion

The functional morphology of male *S. cynipsea* front-leg wing clamps shows the same general pattern found in a previous study of other sepsids (Eberhard 2001*a*). In both *S. cynipsea* and the other species, nearly all of the structures on the male's front legs mesh tightly with the same rigid female wing structures. Thus, on the dorsal surface of the wings of female *S. cynipsea*, four of the five distal setae on the male's front femur meshed with the Stem vein, and two large basal setae on the front femur meshed with the posterior surface of the CuA1 vein (Fig. 3); on the wing's ventral surface, the basal and distal prominences of the male's front tibia meshed with the basicostal and br cells (Fig. 4*c*). The wings of female *S. cynipsea* failed to show coevolutionary morphological adjustments to the male clamps, as they differed from the wings of females of related sepsids (*e.g.*, *Archisepsis*, *Microsepsis*, *Palaeosepsis*; Eberhard 2001*a*) only in minor details, and these differences showed no mechanical association with differences in male morphology.

Differences between male and female *S. cynipsea* and other sepsids (Eberhard 2001*a*) include the convergence of the two long basal setae of the front femur of male *S. cynipsea* when they are inserted into the deep groove just posterior to the CuA1 vein (Fig. 7*d*) (a single large seta is inserted into the same fold in *Palaeosepsis pusio* (Schiner, 1868); no setae are inserted into this fold in *Archisepsis* spp.); the sharp bending of the short medial seta against the wing surface (Figs. 1*b*, 1*c*, 3*c*); the lack of areas that are free of microtrichia on the dorsal Stem vein, where the male's femur makes contact (bare areas occur in all species of *Archisepsis* that have been studied); and a possibly less pronounced

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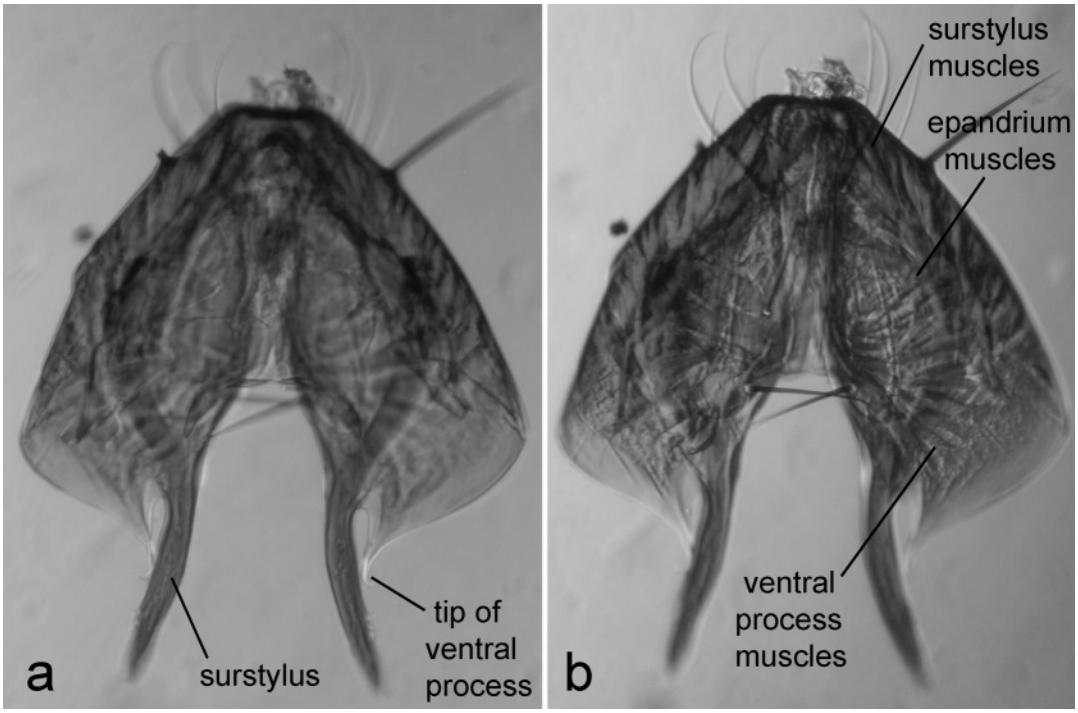
Fig. 8. Dorsal view of the ventral processes of the surstyli of male *Sepsis cynipsea* and their muscles under a compound microscope. (*a*) Focus on tip of right ventral process shows its lack of dark cuticle; (*b*) focus on muscles on the right side shows bands of muscle whose contraction will move the ventral process medially. Other bands of muscle are named for the parts they apparently move when they contract.



depression in this same area of the Stem vein. One further minor difference with respect to species of *Archisepsis* and *Microsepsis* was that the cluster of campaniform sensilla at the base of the ventral surface of the Subcosta vein was partially covered by a projection of this vein (Fig. 4) (a similar, though less prominent, projection also occurs in *P. pusio*). This projection, however, seems unlikely to represent a female defensive structure for resisting male clamps: as in other sepsids, this cluster of sensilla in *S. cynipsea* was not contacted by the male's front tibia when it clamped the female's wing.

In sum, the males of the different genera of sepsids studied to date use quite different structures on their front legs to grasp very similar complex, rigid female wing surfaces. The lack of species-specific female adjustments to species-specific male traits is not in accord with predictions of the lock-and-key or antagonistic morphological coevolution hypotheses. In addition, there are female sense organs in the area grasped by the male in all species, as expected under the female choice by sensory stimulation hypothesis. The distribution of campaniform sense organs in female *S. cynipsea* is approximately the same as that in females of the other sepsid genera on both the dorsal and ventral surfaces of the female's wing near the sites where the male grasps it (Eberhard 2001*a*). These differences show no obvious relationship to differences in male grasping structures, a pattern that has also been seen in the females of other genera. Stimulation of the wing by the male's species-specific grasp induces the female to copulate in two other species of sepsids (Eberhard 2001*a*, 2002*b*).

It might seem that the male-imposed damage to microtrichia on the female's wing, apparently never noted previously in any sepsid species, is an incidental consequence of male grasping ("incidental mechanism damage" of Cordero and Eberhard 2005). But the placement of the medial seta on the male's femur seems to inevitably cause it to rub and perhaps break microtrichia on the female's wing (Fig. 3*c*). It is not clear what advantage a male could derive from producing this damage. These small patches of damage are presumably disadvantageous for the female (otherwise why would she produce

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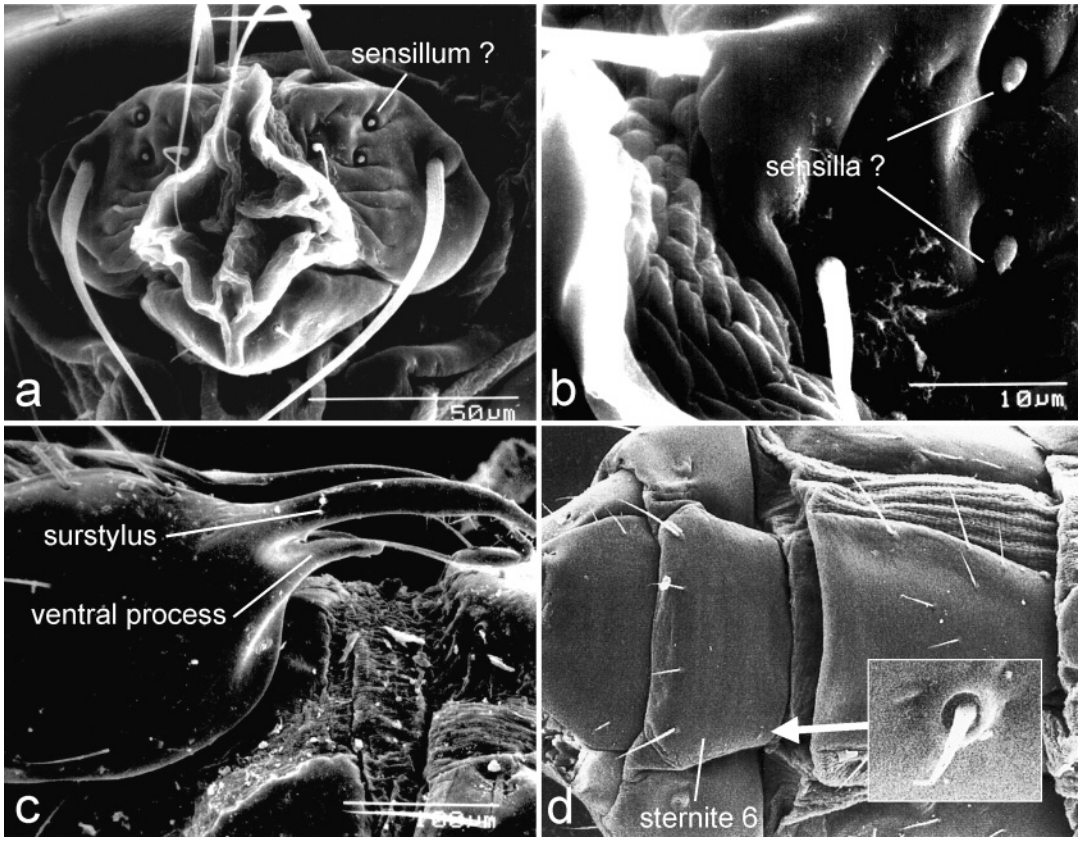
Fig. 9. Terminalia of female and male *Sepsis cynipsea*. (*a*) Posterior view of the proctiger; (*b*) close-up of possible sensilla on female's proctiger; (*c*) lateral view of male surstyli that disengaged from the female during copulation; (*d*) ventral view of female abdomen, showing (inset) the small anterolateral seta on sternite 6 (similar setae occurred on sternites 5 and 7).



microtrichia here?), but the severity of this disadvantage is unclear. The microtrichia in this general area of the wing are not obviously different in structure or density (Fig. 1) and thus give no sign of female coevolutionary adjustments.

Comparative data from other species are similarly inconclusive regarding possible male–female conflict over male-imposed damage to wing microtrichia. In some species of *Archisepsis*, *Palaeosepsis*, and *Microsepsis*, the general area of the wing that is clasped by the male is bare and lacks microtrichia in females, and the bare area is reduced or absent in males (Eberhard 2001*a*; W.G. Eberhard, unpublished data). However, details of the mesh of the male's front leg with the female's wing show that the males of these species do not have structures that would rub the female's wing in ways comparable to the action of the medial seta of *S. cynipsea* (Eberhard 2001*a*; W.G. Eberhard, unpublished data). Similar damage to patches of microtrichia occurs on the wings of female *Themira minor* (Haliday, 1833), *Palaeosepsis dentatiformis* Duda, 1925 (which lack bare areas), and *Archisepsis pleuralis* Coquillett, 1900 (which have bare patches, but males often struggle violently with other males while mounted on females and thus may damage them) (W.G. Eberhard, unpublished data). Details of the male–female mesh in *T. minor* and *P. dentatiformis* are unknown, but males of *P. dentatiformis* lack obvious scraping setae. Thus there is no consistent correlation between damage to microtrichia and scraping setae, nor between damage and bare patches.

The second set of species-specific male structures that contact the female are the genitalia. Once again, the female structures grasped by the male's genitalic surstyli during copulation lack species-specific features that adjust them to the male designs, and thus also fail to

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conform to predictions of the lock-and-key and antagonistic morphological coevolution hypotheses. The only rigid female structure (abdominal sternite 6) that was near the male surstyli during copulation is very simple (Figs. 7, 9*d*). The male surstylus was sometimes near the small anterolateral seta on female sternite 6 (Fig. 9*d*), but there are similar small setae on female sternites 5 and 7, so this seta is apparently not specialized to sense the male's genitalic grasp; it may nevertheless be able to sense it.

These patterns in the designs and functions of male surstyli and female sternites appear to be widespread in other sepsids. The male's species-specific surstyli grasp the same area of the female, her 6th abdominal sternite and the surrounding membranes, in all sepsids that have been observed: *Archisepsis diversiformis* (Ozerov, 1993) and *Archisepsis ecalcarata* (Thomson, 1869) (Eberhard and Pereira 1996); *Microsepsis armillata* (Melander *et* Spuler, 1917) and *Microsepsis eberhardi* Ozerov, 1997 (Eberhard 2001*a*); *P. pusio* and *P. dentatiformis* (Eberhard 2002*a*); *Sepsidomorpha* sp. (Eberhard 2001*b*); *T. minor* (Eberhard 2003); and *Pseudopalaeosepsis nigricoxa* Ozerov, 1992 and *Saltella sphondylii* (Schrank, 1803) (W.G. Eberhard, unpublished data). The female's 6th sternite is apparently very simple and relatively uniform throughout the family; it is nearly featureless in several species in each of *Archisepsis*, *Microsepsis*, *Palaeosepsis*, and *Sepsis* and also in species in other genera (*T. minor*, *P. nigricoxa*, *S. sphondylii*, and *Sepsidomorpha* sp.) (W.G. Eberhard, unpublished data). Despite the diversity and often species-specific differences in male surstylus morphology that are evident in taxonomic publications on species of these groups, female abdominal sternites are consistently missing from lists of species-specific traits in these same studies (Duda 1925, 1926; Pont 1979; Steyskal 1987; Silva 1993; Ozerov 1992, 1993, 1998). Thus the female structures contacted by the male genitalic surstyli also fail to conform to predictions of the lock-and-key and antagonistic morphological coevolution hypotheses.

A second context in which male surstyli may function in *S. cynipsea* is the precopulatory genitalic engagements ("temporary genital contacts" of Parker 1972*a*). These contacts almost certainly do not result in intromission or sperm transfer. The male's intromittent genitalia were not close to the female's vulva; the female's ovipositor was often extended during these contacts, showing that the male had not penetrated her (Ward (1983) interpreted these female movements as a means by which females prevent intromission); and the contacts all ended without any sign of the forceful pulling that accompanies extraction of the intromittent male genitalia from the female in this and other sepsids (Parker 1972*a*; Eberhard and Pereira 1996). Successful copulation in *S. cynipsea* involves transfer of a spermatophore deep in the female's vagina that is similar in size and basic form to that of *A. diversiformis* (Eberhard and Huber 1998; W.G. Eberhard, unpublished observations). In sum, these precopulatory engagements seem most likely to represent courtship (presumably under sexual selection by traditional female choice) or another male behavior designed to increase the chances that the female will raise her abdomen and proctiger and allow the male to copulate (presumably under sexually antagonistic selection) (see below).

Once copulation began, females did not offer any perceptible behavioral resistance to being squeezed by the male's surstyli (or by the small processes at their bases). In addition, the rhythmic squeezes with the surstyli seen in this and other sepsid species seem stimulatory rather than "aggressive", in the sense that they are not appropriate to physically coerce female reproductive decisions. The female structures that are squeezed by the male's surstyli are not associated with the female's internal reproductive tract (Eberhard 2002*c*). A more likely function for such rhythmic genitalic squeezing is stimulation of the female (Eberhard 2001*c*), as expected under traditional female choice.

The functional morphology of the male and female genitalia in *S. cynipsea* resemble that of other sepsids in several other details. As just noted, the female proctiger is elevated during copulation (Fig. 6*b*), as in species of *Archisepsis*, *Microsepsis*, *Palaeosepsis*, *Themira*, and *Sepsidomorpha* (Eberhard and Pereira 1996; Eberhard 2001*b*, 2001*c*, 2002*b*, 2003), and a small mass of clear white material was present near the ventral base of the male's genitalia during copulation (Fig. 6*b*), as in species of *Microsepsis* and *Themira* (Eberhard 2001*d*, 2003). The major difference between species is that male *S. cynipsea* have a unique, small, apparently independently mobile process at the base of each surstylus. No similar structure is known, to my knowledge, in other sepsids (though the muscles in the epandrium of *Sepsis thoracica* (Robineau-Desvoidy, 1830) and

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perhaps *Xenosepsis* sp. suggest that similar structures may exist — see Eberhard 2003). The lateral teeth and the hooked tips of the small ventral processes may be modifications to grasp and (or) stimulate the female. The relative lack of sclerotization of the tips of these processes suggests that they do not grip the female forcefully, favoring (by elimination) the stimulation hypothesis. The musculature of the small ventral processes (and thus their probable ability to move independently) also fits with the stimulation hypothesis. There were no signs of modification of the female membrane in *S. cynipsea* where the ventral processes made contact, arguing against lock-and-key and sexually antagonistic morphological coevolution explanations for this structure.

In sum, the coevolutionary adjustments of female morphology to species-specific differences in the male front legs and genitalic surstyli, which are predicted by the lock-and-key and antagonistic morphological coevolution hypotheses, are missing. An additional prediction of the antagonistic morphological coevolution hypothesis — that female designs should have defensive properties — was also not confirmed. Neither female wing bases nor female abdominal sternites and surrounding membranes showed signs of the defensive properties predicted by this hypothesis. The wing bases have sense organs that might sense stress exerted by the male's front legs, as expected under traditional female choice hypotheses. The male surstyli are also moved rhythmically, as if to stimulate the female. The abdominal sternites of the female lack sensilla to sense the male surstyli (unless the small anterolateral setae function this way), but stress receptors could be present in the nearby membranous areas.

As noted in the Introduction, it is possible to salvage the general antagonistic coevolution hypothesis if females show behavioral rather than morphological traits that are species-specific and designed to resist males, and if the differences in male designs are appropriate and necessary to overcome these differences in female behavior. For instance, some aspects of the design of *S. cynipsea* wing clamps seem likely to make the male's hold especially secure with respect to anterior-posterior slippage on the female's wing, when compared with the clamps of other species that have been studied (Eberhard 2001*a*): femoral setae rest on both sides of the female Stem vein, and two large basal setae are braced in the longitudinal-dorsal fold of the wing. Perhaps female *S. cynipsea* differ from other sepsids by struggling in ways that might cause such slippage (*e.g.*, female rocking forward and backward, or twisting, instead of shaking from side to side). To date, however, the pattern of interspecific behavioral differences that has been described is the opposite. Studies of the behavior of *S. cynipsea* do not mention rocking or twisting movements, but only shaking ("swaying" in Parker 1972*a*; "shaking" in Ward 1983; Blanckenhorn *et al.* 2000). Rocking (along with shaking) does occur in other sepsids (Eberhard 2001*a*). None of the studies of *S. cynipsea* or other species are detailed enough, however, to eliminate the possibility of subtle differences in female behavior.

With respect to male genitalic surstylus differences, alternative explanations based on female resistence behavior are feasible, but not entirely convincing. The surstyli of *S. cynipsea* are relatively long and seem appropriate to "overcome" the female's resistance by bending her abdomen ventrally (Parker 1972*a*) by forcefully lifting her abdomen prior to copulation. Such lifting brings the female's vulva within range of the male's intromittent genitalia, and could thus improve his chances of intromission. Females of other species, such as *A. diversiformis*, also reject males by lowering their abdomens (Eberhard 2001*a*; M. Baena and W.G. Eberhard, unpublished data). It should be noted, however, that coercively raising the female's abdomen with elongate surstyli is not sufficient to achieve intromission: the female must actively cooperate, both by not immediately lowering her abdomen again and by flexing her proctiger dorsally to expose her vulva, if intromission is to occur (Eberhard 2002*b*). As in other sepsids (Eberhard 2002*b*), the male genitalia of *S. cynipsea* lack structures that can physically lift the female's proctiger.

As noted above, there are several indications of a stimulatory function for male surstyli. A stimulatory function is also likely for the surstyli of other sepsids (Eberhard 2001*b*). No female behavioral defenses against these squeezes that might favor male diversity are evident in any of these species. In sum, the hypothesis that the elongate male surstyli of *S. cynipsea* function in forcefully coercing rather than stimulating the female does not explain some of the data. The forceful lifting and stimulation functions are not exclusive alternatives.

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