

Threading a needle with reinforced thread: intromission in *Ceratitis capitata* (Diptera, Tephritidae)

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Abstract—The intromission of the male's long, flexible, threadlike phallus into the female's similarly long, thin, S-shaped vagina in tephritid flies is mechanically challenging. The male of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824), folds the basal portion of his phallus back on itself, inserts the folded portion into the female, and then gradually unfolds his phallus inside her vagina. The site of the fold in the phallus gradually moves distally, deeper into the female, dragging the distal portion of the phallus into her vagina. At the same time, the male pulls the tip of the female's ovipositor distally with his surstyli, straightening her vagina. The male uses his phallopodeme and asymmetrical sytergosternite to periodically clamp and release portions of his phallus that have not yet entered the female. Once the entire distal portion of the male's phallus is inside the female, he unfolds it and then moves its distal tip even deeper, reaching the inner end of her copulatory bursa. Straightening movements of the phallus due to increases in fluid pressure probably supply at least part of the force that drives the phallus into the female.

Résumé—Chez les diptères téphritidés, la pénétration du phallus long, flexible et filiforme du mâle dans le vagin également long, mince et sinueux de la femelle présente un défi mécanique. Le mâle de la mouche méditerranéenne des fruits, *Ceratitis capitata* (Wiedemann, 1824), replie la partie basale de son phallus sur elle-même, insère la partie repliée dans la femelle et déplie ensuite graduellement son phallus à l'intérieur du vagin. Le point de repli du phallus se déplace graduellement vers l'extrémité, de plus en plus profondément dans la femelle, entraînant ainsi la partie distale du phallus dans le vagin. Au même moment, le mâle tire vers l'arrière le bout de l'ovipositeur de la femelle avec ses surstyles, redressant ainsi son vagin. Le mâle utilise son phallopodème et son sytergosternite asymétrique afin de fixer et de libérer périodiquement les parties de son phallus qui n'ont pas encore pénétré dans la femelle. Une fois toute la partie distale du phallus du mâle entrée dans la femelle, le mâle la déplie et insère la pointe terminale encore plus profondément pour atteindre la surface interne de la bourse copulatrice. Les mouvements de redressement du phallus causés par une augmentation de la pression hydraulique fournit probablement au moins une partie de la force qui fait pénétrer le phallus dans la femelle.

[Traduit par la Rédaction]

Introduction

Complex genitalia occur in many arthropods, and in some species extreme female morphologies lead to serious mechanical difficulties for males (Kamimura and Matsuo 2001). Tephritid flies offer an example of such a problem. Intromission is mechanically challenging in many tephritids because the female's vagina is a long, thin, S-shaped tube, a design that allows the female to extend her long ovipositor and lay eggs in sheltered sites. The male's intromittent organ, his phallus, which includes a long, highly

flexible, threadlike distiphallus, must penetrate deep into the female to deposit sperm near the ventral receptacle and the mouth of the spermathecal duct (*e.g.*, Hanna 1938; Eberhard and Pereira 1995; Aluja and Norrbom 2000; Marchini *et al.* 2001; Fritz and Turner 2002). Although there are other flies with an elongate female ovipositor and (or) a long filamentous male intromittent structure (*e.g.*, Psychodidae, Micropezidae, Ottitidae), the mechanism used by the male to thread his genitalia into the female has apparently never been described.

Millions of dollars are spent annually on attempts to control the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824), but

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some aspects of its basic biology remain poorly known. Although recent studies provide greatly improved understanding of events involved in sperm transfer deep inside tephritid females during copulation (Eberhard and Pereira 1995; Marchini *et al.* 2001; Fritz and Turner 2002), the initial stages of the process of intromission have apparently never been carefully studied. The only published description of the initiation of intromission in this species is that of Hanna (1938), who claimed that the phallus "...becomes rigid, then...is introduced into the genital pore of the female and pushed into the vaginal duct...". But this description is surely wrong. The phallus is on the order of 40% of the length of the male's body, so a maneuver such as that described by Hanna would have to begin with the tip of the male's abdomen almost an entire abdomen's length from the tip of the female's ovipositor. This position is never observed in mounted pairs (Eberhard and Pereira 1995). Just prior to intromission, the ventral surface of the male's abdomen is pressed against the tip of the female's abdomen, and the sclerite at the tip of her ovipositor (the aculeus) is firmly clasped between the male's surstyli; the tip of his phallus (the glans) lies near the entrance to the vagina. Eberhard and Pereira (1995) intimated (incorrectly, as will be shown below) that the glans then enters her directly.

The aspects of male and female anatomy described in previous publications (Hanna 1938; Feron 1962; Eberhard and Pereira 1993, 1995; Briceño *et al.* 1996; White *et al.* 2000; Marchini *et al.* 2001) that are critical to the events described below are the following. Males attract and court females chemically, and during courtship they inflate both rectal and pleural membranes that are thought to release pheromones. The first step of copulation occurs when the male seizes the distal, rigid tip of the female's aculeus near its tip in a powerful, six-pronged clamp formed by his surstyli. This grasp, in which the apical tip of the aculeus projects into a pocket formed in the ventral intersegmental membrane between the male's syntergosternite and his epandrium just ventral to his surstyli, is maintained apparently unchanged throughout copulation. The male's phallus arises ventrally and consists of three sections: a short basiphallus; a flexible, very long, threadlike distiphallus that is more heavily sclerotized on its ventral side; and a

structurally very complex, mostly rigid glans at the tip of the distiphallus.

In living flies, the distiphallus curls spontaneously toward its membranous side (Eberhard and Pereira 1995). The distiphallus and glans are capable of limited movements. When teased free of the membrane in a live, decapitated male, the distiphallus rhythmically straightened partially about twice per second; each movement occurred in synchrony with an inflation of the basal lobe of the glans (= the "spoon-like structure" of Marchini *et al.* 2001) and a partial straightening at the glans–distiphallus junction (Eberhard and Pereira 1995). These movements were probably produced by increases in fluid pressure within the phallus, presumably due to activity of the ejaculatory apodeme and the sperm sac ("erecting and pumping organ" of Hanna 1938); movements ceased when the basal lobe of the glans was punctured (and liquid emerged) (Eberhard and Pereira 1995).

The female's long, thin, posterior vagina runs in a more or less straight line anteriorly through her sclerotized aculeus and then folds back on itself in an S shape inside the eversible membrane, which lies immediately anterior to the aculeus; both of these structures are partially enclosed in the wider, more basal oviscape (= syntergite). More anteriorly, the vagina extends in a more or less straight line, ending at the copulatory bursa or anterior vagina (Marchini *et al.* 2001). When the female extends her ovipositor, the S-shaped fold in her vagina is straightened.

Materials and methods

A total of 42 pairs of virgin flies of an approximately 4-year-old mass-reared strain derived from wild flies in Costa Rica were frozen with ethyl chloride spray during the first 2 min after the male mounted the female. Each pair was immediately preserved in 80% ethyl alcohol at room temperature. Some pairs remained coupled, while others fell apart. Eight pairs were processed with standard procedures (Eberhard and Pereira 1995) for examination in a scanning electron microscope; the other 34 were carefully observed and dissected or manipulated to ascertain the position of the entire distiphallus. Those in which at least part of the distiphallus and the glans were outside the female were carefully pulled apart to determine the positions of all parts of the male's phallus. In pairs in which the glans was inside the

Fig. 1. Schematic drawings of male genitalia of *Ceratitis capitata* during intromission (female removed in all except *h*). Prensissetae of surstyli are black, phallus (basiphallus, distiphallus, and glans) is densely stippled, syntergosternite is sparsely stippled, and process of the phallapodeme is cross-hatched (saw-toothed regions are cutaway rectal epithelium). (*a*) Rear view of resting position, with glans embedded in the intersegmental membrane anterior to the syntergosternite and the process of the phallapodeme clamping the distiphallus in the membrane to the right of the right surstylus. (*b*) Rear view with glans and distal portion of distiphallus free, with the process of the phallapodeme clamping the distiphallus. (*c*) Similar to *b* but with phallapodeme process raised. (*d*) Ventrolateral view with basal portion of the distiphallus folded 180°; the process of the phallapodeme is not clamping the distiphallus, which nevertheless is in a position to be clamped. (*e–g*) Lateral (*e*, *f*) and posterior (*g*) views of gradual distal movement of fold in distiphallus. In *g* the distiphallus is clamped by the process of the phallapodeme. (*h*) Posterolateral view in which the female was left in place. Only the distal portion of the glans remains to the right of the right surstylus; the fold in the distiphallus is deep inside the female (the male's left surstylus has opened and is not clamping the aculeus).

female, the female was dissected to determine the positions of all parts of the male's genitalia. Inflation of the male's pleural abdominal membranes and rectal epithelium was evaluated relative to that in courting and solitary males. Morphological terminology follows that of White *et al.* (2000) where possible.

Results

The male's syntergosternite, which played an important role in intromission, formed an asymmetrical U-shaped collar just anterior to his epandrium, with its lower tip lying just to the right of his right surstylus. Its tip had a notch (visible only from some angles) between a longer inner extension and a shorter, thicker outer prolongation (Figs. 1*c*, 1*d*). The phallapodeme had a long process whose tip was also slightly notched (Figs. 1*f*, 1*h*) and that fit into the notch in the lower tip of the syntergosternite (Fig. 1*h*). The cuticle was thickened along the posterior edges of both the notch in the lower tip and the phallapodeme process, where they overlapped.

At rest, the distiphallus folded back on itself sharply at its base where it joined the basiphallus, and curved anteriorly and dorsally to the right; it passed under the right surstylus and then dorsally and to the right (viewed from the rear). More distally, it curved in a counterclockwise loop that passed over the male's dorsal midline, then turned back 180° to the right; the distal portion of the distiphallus and the glans were embedded in a fold in the intersegmental membrane between abdominal tergite 5 and the syntergosternite (segments 6–8) (Fig. 1*a*).

As expected from previous observations (Eberhard and Pereira 1993), there was little correlation between the length of time the male

had been mounted and the stage of intromission. In one pair, for example, the entire distiphallus was inside the female after only 30 s, while in other pairs it had not even begun to be introduced after 2 min. Therefore, I did not use the time elapsed to deduce the sequence of events in intromission, but instead used the intermediate positions of the male genitalia between the known starting position of the phallus prior to copulation (glans and distal portion of distiphallus embedded in the male's intersegmental membrane; Fig. 1*a*) and its end position during most of copulation (distiphallus more or less straight, running the length of the vagina, with the glans at the inner end of the copulatory bursa) (Eberhard and Pereira 1995; Marchini *et al.* 2001). In all pairs described below, the male's surstyli had already grasped the distal portion of the female's aculeus. In all but two pairs in the final stage, the female's aculeus was everted at least partially from its normal resting position inside the eversible membrane. In the most extreme cases, the base of the aculeus had emerged completely free of this membrane. The more extreme eversions presumably resulted from the repeated strong posterior pulling movements made by males early in copulation after clamping the tip of the aculeus with their surstyli (Eberhard and Pereira 1995). The positions of the male genitalia, in their presumed order of occurrence, were grouped into six more or less arbitrary stages.

(1) **Glans in fold of intersegmental membrane** (Fig. 1*a*) (8 pairs, 7 dissected). In all but 1 of these 8 pairs, the glans and distiphallus were in their resting positions (above). In the other pair, both the basiphallus and the basal portion of the distiphallus had moved to project directly anteroventrally, but the glans was still in the folded intersegmental membrane. The

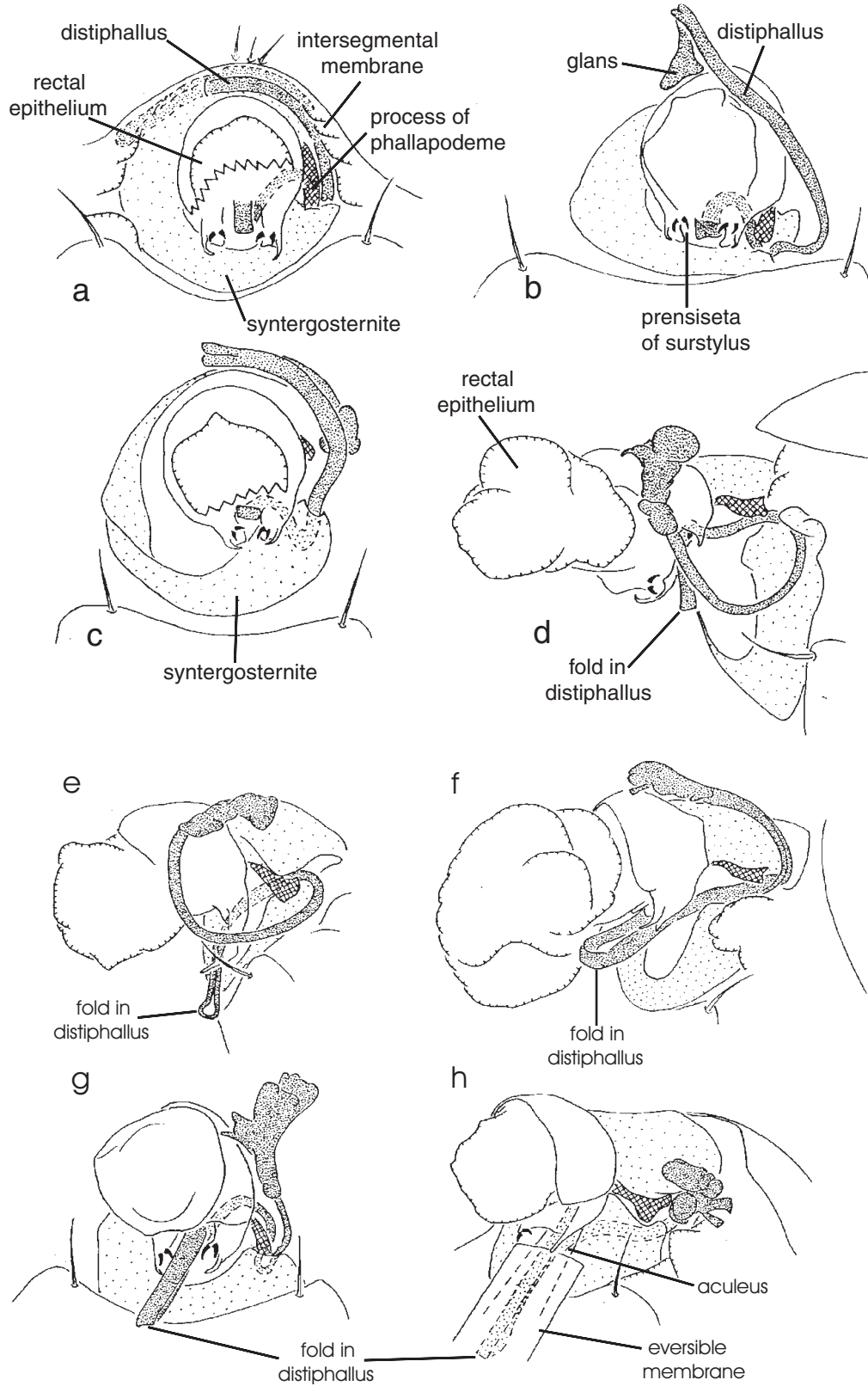
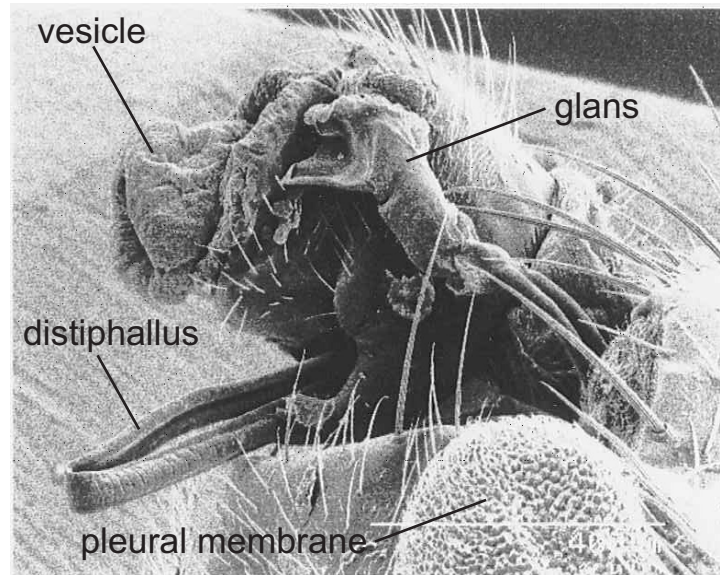


Fig. 2. Scanning electron micrograph of lateral view of male genitalia of *Ceratitis capitata* in stage 4 of intromission (see text), with the glans near the right surstylus (female removed).



mesh of the distiphallus with the phallapodeme process and the lower tip of the synergosternite varied, suggesting that the first steps in freeing the male phallus from the intersegmental membrane occurred in this area. In half of the pairs, the long process on the phallapodeme was inserted deep into the membrane to the right of the right surstylus, trapping the distiphallus against the notched dorsal margin of the ventral arm of the synergosternite, pressing the distiphallus deep into this indentation in the membrane. In the other pairs, this extension of the phallapodeme was partially or completely raised out of this depression in the membrane and did not press forcefully on the distiphallus (Fig. 1*a*) (in 2 males it contacted the distiphallus without deflecting it). In half of the males both rectal and pleural epithelia were still inflated, while in the others they were partially or completely deflated.

(2) **Glans free of membrane, 180° fold in distiphallus not yet established** (Figs. 1*b*, 1*c*) (13 pairs, 11 dissected). The distal portion of the distiphallus and the glans had pulled free from the fold in the intersegmental membrane and had curled dorsally, free in the air (Figs. 1*b*, 1*c*). The basiphallus was deflected ventro-anteriorly, and the sharp 180° fold between the basiphallus and the distiphallus was either reduced (90° in 1 male, 60° in another) or gone altogether (all others). In one pair the distiphallus was clamped by the process of the

phallapodeme (Fig. 1*b*). In another, the distiphallus was still inserted deep into the membrane at the right of the right surstylus and aligned with the notch at the tip of the phallapodeme process, but the process was raised clear of the membrane (Fig. 1*c*), suggesting that the clamp had just been opened but that the distiphallus had not yet straightened itself. The epandrium of this male was rotated counterclockwise (seen from the rear), suggesting that the phallapodeme process may be raised with a twisting motion. In the other 9 males, the distiphallus was free of the phallapodeme. In 6 of the males in stage 2, the pleural membranes were still inflated; the rectal epithelium was either partially (5) or completely (5) deflated in all males.

(3) **Basal portion of distiphallus folded 180°** (Fig. 1*d*) (5 pairs, 3 dissected). A ventral 180° fold had reappeared, but near the base of the distiphallus (Fig. 1*d*). The membranous side of the distiphallus was on the outer side of this 180° fold (Fig. 2). The tip of the doubled portion of the distiphallus was just ventral to the prenisetae of the surstyli and was either at the mouth of or barely inside the female's cloaca (this detail could not be resolved because the pairs had fallen apart). The more distal portion of the distiphallus ran to the right and dorsally from under the right surstylus and was, along with the glans, free in the air (Fig. 1*d*). The distiphallus was not clamped by the phallapodeme. The

male's pleural membranes were inflated in all pairs, while the rectal epithelium was partially (1) or completely (2) deflated.

(4) **More distal portion of distiphallus folded 180°, glans outside female** (Figs. 1e–h, 2, 3) (6 pairs, 4 dissected). The site of the 180° fold in the distiphallus gradually moved distally and was located deeper and deeper in the female. The glans had not yet entered the female and was to the right of and above the right surstylus. In most pairs, the distiphallus emerged from under the right surstylus (Fig. 3) and was clamped by the phallapodeme process and the tip of the synergosternite (Fig. 1g). In the most advanced pair (Fig. 1h), most of the distiphallus was inside the female, and only the glans remained in the clamp. In 3 of 4 pairs, the male's pleural membranes were inflated; his rectal epithelium was partially (1) or completely (3) deflated.

(5) **Glans inside female and folded back on extended distiphallus** (4 pairs, all dissected). The distiphallus was more or less straightened. The glans was in the posterior vagina of the female and was folded back toward the sclerotized side of the distiphallus at an angle that varied between 90° and 180° (Fig. 10 in Eberhard and Pereira 1995). The female's posterior vagina was still extended so that the S-shaped fold was at least partially straightened. In 3 of 4 males, the pleural membranes were inflated; the rectal epithelium was partially (1) or completely (3) deflated.

(6) **Glans inside female and unfolded from distiphallus** (4 pairs, all dissected). The entire distiphallus was straightened inside the posterior vagina, and the glans was unfolded and its distal tip projected anteriorly, deeper into the female (Fig. 1 in Eberhard and Pereira 1995). In two pairs, the female's vagina (and the distiphallus within) had a distinct S-shaped fold, and in one of these the glans was in the anterior vagina of the female. The male pleura were still inflated in only 1 of 4 pairs, and the rectal epithelium was completely deflated in all of them.

Discussion

A tentative description of the process of intromission can be deduced by combining the observations described here with previous descriptions (Eberhard and Pereira 1995). The male first firmly grasps the female's aculeus with his surstyli. Then, after moving his phallapodeme

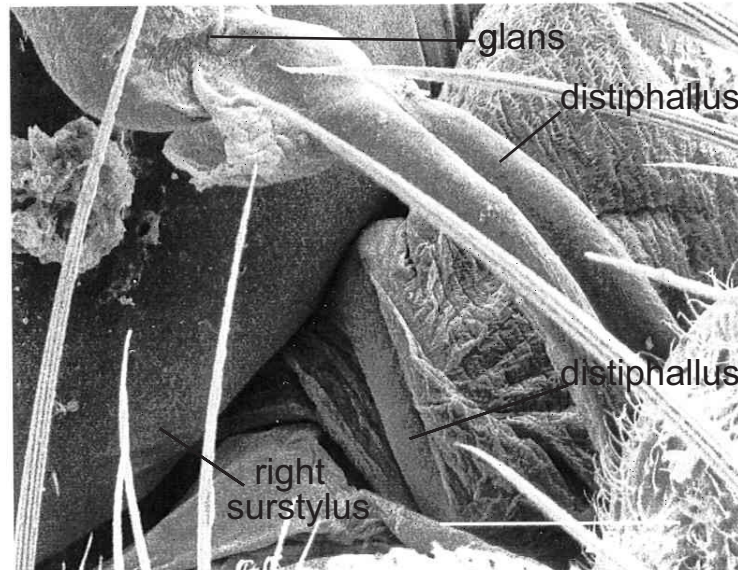
process to unclamp his distiphallus, he removes his glans and distiphallus from their resting positions in a fold of the intersegmental membrane (possibly by increasing fluid pressure or by brief inflation of this membrane). During this process, the sharp, nearly 180° bend at the junction between his basiphallus and his distiphallus is straightened. This straightening can occur just before the glans is lifted free, but perhaps more often just afterward. The distiphallus is then folded 180° near its base, perhaps where it contacts the cloacal opening on the female's aculeus. At the same time, a more distal portion of the distiphallus is again clamped between the phallapodeme process and the lower tip of the synergosternite. The notches at the tip of the phallapodeme process and the ventral tip of the synergosternite probably facilitate clamping of the distiphallus.

The mechanism by which the male folds his distiphallus is not certain. Because his surstyli are gripping the female's aculeus throughout copulation, they must be immobile and thus cannot be involved directly. Folding might be accomplished by movements of the distiphallus or perhaps the phallapodeme process. Perhaps by increasing the fluid pressure within his distiphallus as it presses against the female, or by shifting the grip of his phallapodeme process on the more distal portion of the distiphallus, the male may cause the basal-most portion of his distiphallus to fold back on itself. The pulling and thrusting movements of the male's abdomen that pull the female's aculeus from the eversible membrane and cause her ovipositor to fold or buckle briefly (Eberhard and Pereira 1995) constitute another possible mechanism for folding the distiphallus.

In any case, the folded distiphallus enters the female. It then gradually penetrates deeper as the point of folding moves distally along the distiphallus. The distal movement of the fold could be driven by straightening movements of the distiphallus, movements of the clamp with the phallapodeme process, or thrusting. It seems certain that the clamp formed by the phallapodeme process must open periodically to enable the male to gradually introduce the more distal portions of his distiphallus into the female.

By the time the fold in the male's distiphallus has reached the base of the female's aculeus, only the distal portion of his distiphallus and his glans remain outside the female. They are just to the right of the right surstylus, and the

Fig. 3. Closeup view of distiphallus of *Ceratitis capitata* emerging from under the right surstylus and then folding dorsally and to the left.



distiphallus is clamped by the phallapodeme process (Fig. 1g). The glans is then pulled into the cloacal opening and through the female's entire aculeus. This process apparently occurs rapidly, because intermediate steps were not represented in any of the pairs. The apparent rapidity of this movement is surprising, as it would seem to be the most mechanically difficult part of intromission. The much wider glans must be dragged through the small cloacal opening in the aculeus and then through the thin vaginal tube inside the relatively rigid walls of the aculeus. Perhaps when the fold in the distiphallus has passed beyond the base of the aculeus and into the much larger, less constricted space within the female's eversible membrane and her capacious sytergite, the straightening movement of the distiphallus becomes less constricted and more powerful and can drag the glans rapidly through the aculeus.

The glans is folded back on the distiphallus when it reaches the portion of the vagina in the female's sytergite just distal to the vaginal sclerites (stigma) (e.g., Figs. 10, 11 in Eberhard and Pereira 1995). This folded position accords with the hypothesis that the force pulling the glans and the distal portions of the male's distiphallus into the female is the unfolding of the distiphallus. The glans was not folded back onto the distiphallus when it was pulled into the female's aculeus, as the glans trailed behind the tip of the distiphallus rather than being folded

against it just before it entered the aculeus (Figs. 1h, 3). Probably the glans became folded back on the distiphallus after it reached approximately the basal end of the aculeus and was then propelled still deeper into the female by straightening movements of the distiphallus. A straightening movement of the distiphallus in this less confined portion of the vagina (inside the eversible membrane) could cause the tip of the distiphallus to drag the glans after it, thus producing the fold at the glans–distiphallus junction.

The final stage of intromission has been documented previously (Eberhard and Pereira 1995). With the distiphallus more or less straight, the glans straightens itself at its base, orienting its tip toward the copulatory bursa. Subsequent rhythmic pushing movements of the basal lobe of the glans (Eberhard and Pereira 1995) propel the glans and the tip of the distiphallus deeper, until the distal tip of the glans reaches the inner end of the female's copulatory bursa, where sperm are deposited (Marchini *et al.* 2001).

During the process of intromission (and also later in copulation), the male repeatedly pulls the female's aculeus posteriorly by moving his entire abdomen dorsally and rearward (Eberhard and Pereira 1995). I did not determine whether these external movements were coordinated with internal events, but it is clear that they must straighten the S-shaped fold in

the vagina and thus probably facilitate penetration of the phallus. In some cases the base of the aculeus was pulled completely from its usual telescoped position inside the eversible membrane.

The male's pleural membranes sometimes remained inflated late in the process of intromission, so it is possible that females continued to receive chemical courtship cues from the male during intromission. However, these membranes were deflated in a substantial proportion of males even very early in intromission (stage 1), suggesting that inflation may not be functionally important during intromission.

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