COURTSHIP BEHAVIOR OF MALE *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE) IN CAPTIVITY

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ABSTRACT

Video recordings of *Ceratitis capitata* courtship behavior revealed several hitherto unreported details. The droplet of pheromone at the tip of the male's abdomen during wing vibration was partially or completely retracted during wing buzzing and head rocking. Wing vibration gave way to wing buzzing and head rocking during the last 1-2 sec before the male attempted to mount the female; the male ceased rocking his head during the last 0.3 sec before a mounting attempt. Immediately after landing on the female, the male performed up to three additional types of apparent courtship before achieving intromission. The circumstances in which males attempted to mount females differed from those in which males abandoned courtship: the male was closer to the female and the two flies were oriented more directly toward each other. Neither reductions in distance nor more precise orientations appeared to be the immediate cues releasing mounting attempts, however.

Key Words: Medflies, *Ceratitis*, courtship, behavior.

RESUMEN

Video grabaciones del comportamiento de cortejo de *Ceratitis capitata* revelaron varios detalles antes desconocidos. La gotita de feromona que aparece en la punta del abdomen del macho durante la fase de vibración de las alas fue resorbida cuando diò inicio la fase de zumbido de las alas y balanceo de la cabeza. La vibración de las alas fue reemplazada por el zumbido de las alas y el balanceo de la cabeza durante los últimos 1-2 segundos antes de que el macho intentara montar a la hembra; la oscilación de la cabeza terminó 0.3 segundos antes del intento de montar a la hembra. Una vez sobre la hembra, el macho ejecutó hasta tres tipos de cortejo aparente antes de voltearse e intentar copularla. Las circunstancias en las cuales los machos intentaron montar a las hembras difirieron de aquellas en las cuales los machos abandonaron el cortejo: el macho estaba más cerca de la hembra y las dos moscas estuvieron más directamente orientadas una hacia la otra. Ni la reducción en la distancia entre las moscas ni una orientación más precisa parecieron ser las señales inmediatas que liberan la conducta de la monta.

The basic nature of courtship and copulation behavior in the medfly, *Ceratitis capitata* (Wiedemann) is well known. Males mate in leks, in which males behave territorially and each occupies a separate leaf, and at oviposition sites where territorial behavior is reduced (Prokopy & Hendrichs 1979, Hendrichs & Hendrichs 1990, Whit-

tier et al. 1992, Shelly et al. 1993). In leks, and less often when alone, males release a long distance attractant pheromone, exposing a droplet of liquid by everting a balloon-like structure formed by a membranous portion of the rectal epithelium (Arita & Kaneshiro 1986). Male courtship begins when a female approaches a male, and includes the following behavior patterns (Feron 1962): (1) continuous wing vibration, often performed while the male is facing the female and the tip of his abdomen is bent ventrally with the pheromone droplet present on the everted rectal membrane ("vibrate wings", or stage II of Feron - 1962) (a plume of pheromone is probably thereby wafted toward the female); (2) wing buzzing, during which the wings are rhythmically moved forward and back ("buzz wings"); and (3) rapid rotations of the head ("head rock"). Wing buzzing and head rocking often occur simultaneously (stage III of Feron 1962; see also Rolli 1976 on the resulting songs).

After courting the female, the male leaps onto her and at least sometimes buzzes his wings. These vibrations may serve as further courtship (Zapien et al. 1983), or to maintain the male's balance while he positions himself to intromit. A recently discovered courtship behavior sometimes occurs after the male has mounted the female but before he has achieved intromission. The male nips the female at the tip of her abdomen with his genitalic surstyli. This is often followed by her extending her aculeus, which allows him to clamp it with his surstyli so he can intromit (Eberhard & Pereira 1993).

Presumably most of these male behavior patterns serve to elicit crucial female responses: to approach, or to stop and allow the male to approach close enough that he can mount; to align her body properly for a mounting attempt; and to subsequently allow him to intromit. More often than not, however, courtship fails to result in female acceptance. For instance, the female walked away from a courting male in more than 90% of courtships in the lab (Whittier et al. 1994); and the female dislodged the male after he had mounted in more than 90% of mounting attempts (Kaneshiro 1991) (see also Feron 1962). Thus certain male behavior patterns, and appropriate transitions from one type of courtship behavior to another, may have important effects on a male's chances of copulating.

The role of visual stimuli from the female in eliciting wing vibration (stage I of Feron 1962) and of olfactory stimuli from other males in eliciting pheromone release have been demonstrated experimentally, though only with qualitative data (Feron 1962). Many other important aspects of the coordination of male behavior remain unstudied, however. For instance, there are apparently no studies of the factors that may affect male decisions such as whether to turn toward the female, to advance toward the female, to shift from wing vibration to wing buzzing, to rock the head, or to attempt to mount. Decisions by females (e.g. whether to leave, to allow the male to approach, to allow him to mount, to extend the aculeus and allow intromission) are also unstudied.

Understanding these sorts of details may have important practical consequences. The medfly is a notorious agricultural pest, and large sums of money are spent annually in raising and releasing sterile males to mate with wild females. A better understanding of the details of medfly courtship may have important implications in attempts to maintain the competitive quality of mass-reared males in the face of possible differences in selective regimes in the field and in mass-rearing facilities (e.g. Spates & Hightower 1967, Boller et al. 1981, Kaneshiro 1991), as well as substantial variations in some behavioral aspects such as songs (Rolli 1976).

This paper attempts to lay the groundwork for such studies by describing the details of male courtship behavior. These descriptions permit preliminary conclusions regarding the cues used by males to trigger attempts to mount females.

MATERIALS AND METHODS

Three recordings of 0.5 h each were made of a strain of flies that had been mass-reared for 14 years at the Organización Internacional Regional de Sanidad Agropecuaria (OIRSA) in San José, Costa Rica using a National "Omnipro" video camera (30 images per s). All events were taped which occurred in an area of 588 cm^2 on the cloth wall of the cage through which females oviposited, and the tapes were then studied to follow individual males during courtship sequences. The density of flies resting on this cloth in 20 counts separated by 5 min, averaged 0.55±0.083 flies per cm^2. Supplementary recordings of both wild flies and of a strain of flies that had been mass-reared for 4.5 years were used to clarify some behavioral details. Unless stated otherwise, all data are from the 14 year strain.

Data taken from the recordings included the distance of the male from the female (measured on screen of the monitor and later converted to cm), the angle of male orientation toward the female (male orientation), and the angle of alignment between male and female (female orientation) (Fig. 1). Male behavior (turn, walk forward or backward, vibrate wings, buzz wings, rock head, leap onto female) was also noted. All drawings are based on images traced from videotapes; body parts (legs, wings, etc.) that were out of focus or otherwise unclear were omitted. All averages are followed by one standard deviation. The flies were not marked, thus it was not certain that different courting pairs involved different individuals. The cage contained over 10,000 flies, however, so it is very likely that each male seen courting was a different individual.

RESULTS

The most common sequences of behavior (Fig. 2) were similar to those reported by other authors (e.g. Feron 1962). The following details, however, were different.

(A) Resorption of the pheromone droplet. As has been described by Feron (1962), males consistently bent their abdomens to hold the everted pheromone droplet ventrally while they vibrated their wings (Fig. 3). When wing buzzing replaced wing vibration, however, the droplet was usually resorbed (completely resorbed in 75% of 12 sequences, 25% partially resorbed in the others).

(B) A burst of rapid forward-backward rocking of the male's body after he landed on the female (Fig. 4). Small details of the flies' movements during body rocking, such as deflections of the female's wings, made it clear that the male rather than the female produced these movements. In a sample of 15 mountings from the 4.5 year strain, the time elapsed between landing and the beginning of body rocking averaged 0.43±0.31 s, the number of rocking movements averaged 4±2.1 per mount (range 1-7), and the duration of body rocking averaged 1.64±0.23 s. Rocking movements of this sort are not a normal part of landing behavior and were never observed in flies landing on the wall of the rearing cage.

(C) Cross-legged raising of the female oviscape and

(D) Rubbing on the ventral surface of the female abdomen with the male's hind tarsi and tibiae. In at least those pairs in which the male had difficulty intromitting, he repeatedly raised the tip of the female's abdomen and rubbed its ventral surface with his hind legs (Fig. 5). In one wild pair in which more than 5 min of this behavior was taped before the male finally achieved intromission, some movements of the male's legs were produced by pushing movements of the female's hind legs; nevertheless the male clearly rubbed her on other occasions when her legs were immobile.

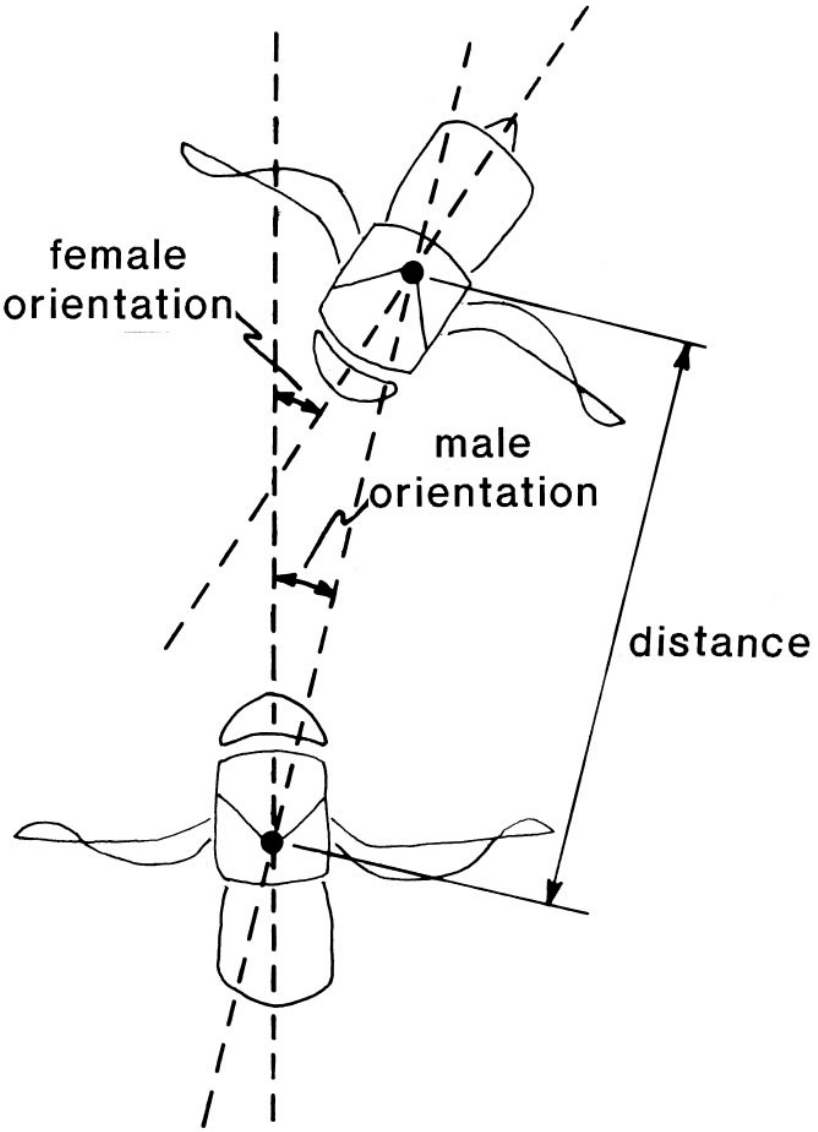

Figure 1. Distances and angles measured from video recordings.

Slow motion analyses of video recordings also revealed new details of some previously described behavior patterns. Wing vibration involved both rapid vibration (too fast to be resolved in 30 frames per s images), and slower, small deflections from side to side about 5-6 times per s (Fig. 6). Wing buzzing included two different components: a rapid continuous vibration of the wings (too fast to be resolved in 30 frames per s im-

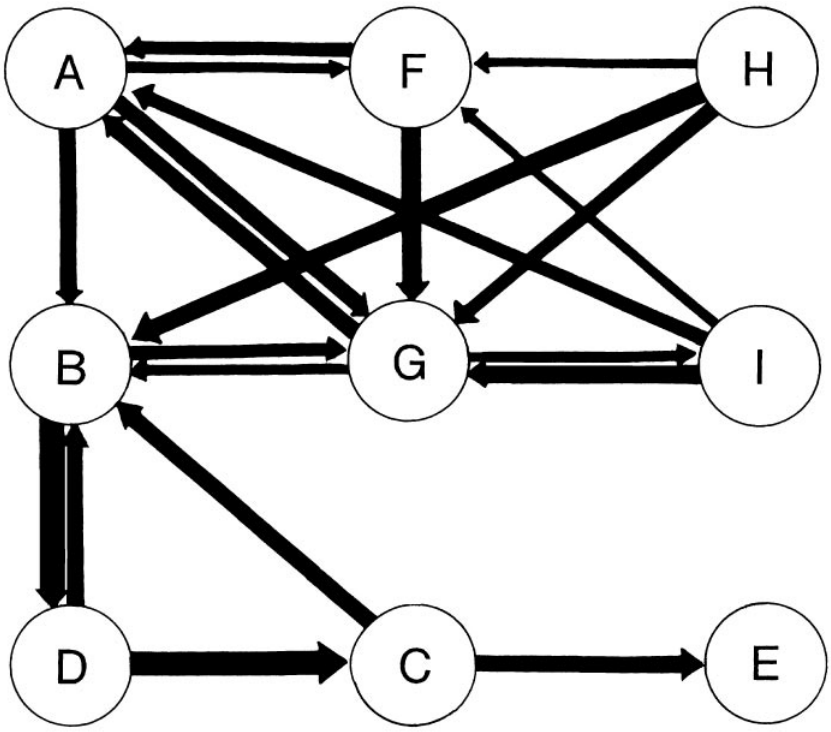

Figure 2. Frequencies of transitions between different male behavior patterns in 17 courtship sequences in which the male attempted to mount the female. Widths of arrows are proportional to frequencies. A = reorient; B = vibrate wings; C = buzz wings; D = rock head; E = mount female; F = walk toward female; G = immobile; H = move away; I = rub legs together.

ages); and a rapid, repeated forward-backward movement about 3-4 times per s (average 1/0.26±0.11 s, N=34) (Fig. 7). The number of forward-backward movements per buzz averaged 10.15±9.95 (N=34). Head rocking also involved more complex movements than previously appreciated. As described by Feron (1962), the largest displacements of the male's head were rotatory (A in Fig. 8); but lateral as well as dorsoventral movements also occurred (B and C in Fig. 5). Rotation in one direction (e.g. to the left) was nearly always followed by rotation in the other direction. Rotations were too rapid to allow precise measurements of angles, but it appeared that the first rotations in a series tended to be larger than later ones. The frequency of rotations averaged 24.6±22.6 per s in 21 courtships.

Possible stimuli triggering male mounting attempts were checked by analyzing details of the courtship behavior of 17 pairs during the 6 s period immediately preceding the male's leap onto a female that was more or less facing him (3 other leaps from behind the female were excluded). The data in the three images during each lapse of 0.1 s were averaged during the second immediately preceeding the leap; similar averages were calculated for each of the 0.3 s during the preceeding 5 s. As shown in Fig. 9A, wing vibration was the earliest courtship behavior in all interactions, occurred at

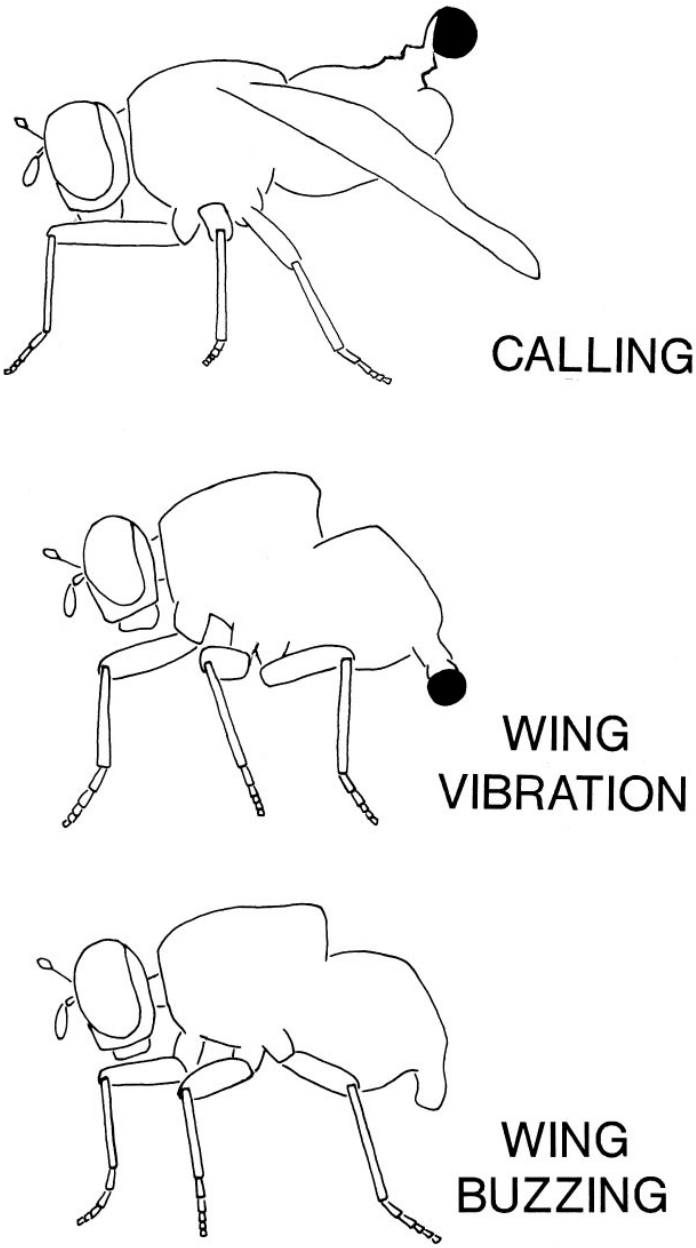

Figure 3. A droplet of presumed pheromone (black) was exposed dorsally by calling males, and the droplet was directed ventrally during wing vibration. The droplet was usually resorbed when the male began wing buzzing. The drawing at the bottom represents an image 0.4 sec. after the drawing in the middle.

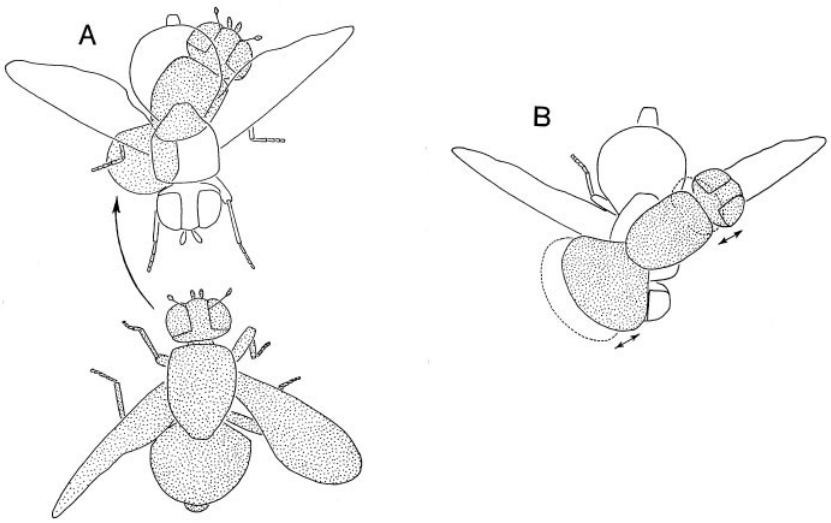

Figure 4. After landing on a female (left; the upper image of the male followed the other by 0.06 s), the male rocked his body briskly anteriorly and posteriorly (right; the dotted lines in the figure represent the male's position 0.03 sec after the solid lines) (drawings of wild male and female; male wings omitted for clarity).

intermediate frequences during the last seconds before a leap, and was replaced completely by buzzing during the last second. Head rocking was absent >5.8 s before the leap; it became especially common about 1 s before the leap, and then disappeared abruptly 0.3 s before the leap.

The male remained oriented toward the female and showed no clear trends during the 6 s preceding a leap, other than a possible decrease in the variance during the final s (Fig. 9C). The angle of the female's alignment toward the male was also relatively constant and variance declined head-to-head during last s before the jump (Fig. 9B). The average distance between the two flies was also nearly constant during the entire courtship period, decreasing slightly and becoming less variable just before the leap (Fig. 9D).

Using the same 17 interactions, male and female behavior were analyzed as a function of the distance between the two flies during the 6 s preceeding a leap. The likelihood that a leap would occur was greater when the flies were closer, although in a few cases males leapt from 0.75-1.0 cm away (Fig. 10A). The relationships between distance and the different male courtship behavior patterns were similar to those in Fig. 9. Wing vibration was more common farther from the female, and wing buzzing and head rocking more common closer to her (Fig. 10B). Similarly, male orientation was low and nearly constant at different distances, while female alignment was closer to 0° at shorter distances. Males were more likely not to be walking than females at all distances, and both males and females were more likely to be immobile when they were closer together (Fig. 10C).

These analyses suggest that neither male or female orientation nor the distance between the flies is the immediate cue triggering mounting attempts by males. These factors are, nevertheless, probably important in predisposing males to attempt to mount, as shown by comparisons between the relative positions between males that

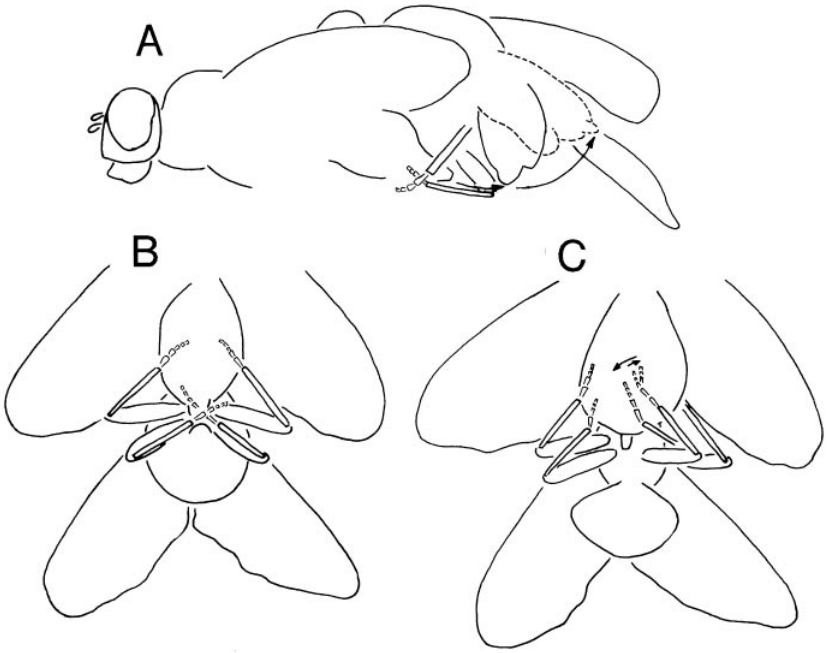

Figure 5. Behavior of a mounted male attempting to achieve intromission. A. Male moves his abdomen dorsally after pressing his genitalia against those of the female, but having failed to clasp her aculeus with his surstyli. The tip of the male's abdomen was touching that of the female 0.3 s before the position indicated by solid lines; 0.3 s later it was in the position indicated by dotted lines. B. Mounted male raises the tip of the female's abdomen with his crossed hind legs as he bends his abdomen ventrally and anteriorly to bring his genitalia into contact with her. C. The male rubs the ventral surface of the female's abdomen with one hind tarsus, which first moves anteriorly (dotted lines follow solid lines by 0.1 s), and then posteriorly (0.2 s later).

they attempted to mount, and males that stopped courting without being interrupted. Males which abandoned courtship attempts were farther from the female (0.79±0.38 as compared to 0.45±0.23 cm), were less oriented toward the female (42.3±30.0 vs. 4.3±6.2°), and the female was less aligned toward the male (88.9±52.7 vs. 14.4±15.2°) (N=35 for abandoned attempts) (all $p<0.01$ with Mann-Whitney U Test).

When the overall averages for the behavior of a random sample of males seen courting were compared with averages for courtship sequences in which the male attempted to mount the female, the durations of wing vibration but not wing buzzing or reorientation movements by the male head rocking were shorter for courtships preceding mounting attempts (Table 1). Thus, if a male was going to mount a female, he tended to do so relatively rapidly. The possible importance of rapid male mounting was illustrated by the fact that most courtships in mass-rearing cages were interrupted when another fly walked or landed nearby. Of 30 randomly chosen cases in which a male began courting a female, 63% were interrupted by another fly. Of 29 cases in which a male was interrupted, in only 17% did he resume courtship of the same female.

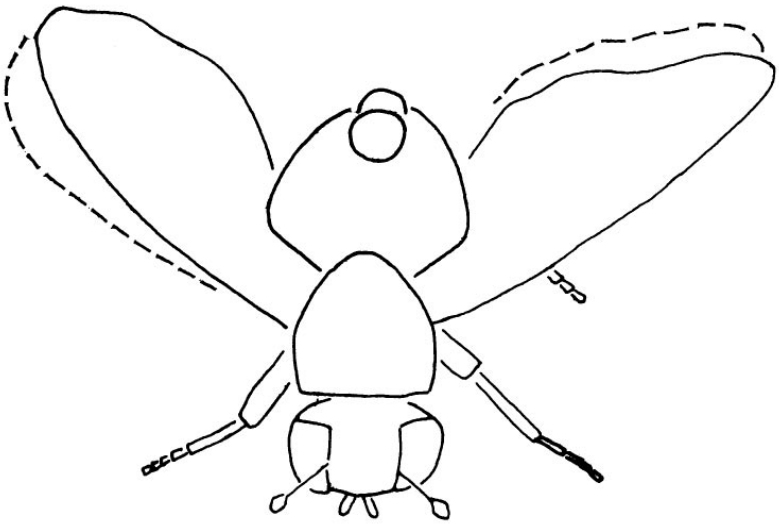

Figure 6. Wing vibration involved both rapid vibrations (area covered by displacements indicated by solid lines) and slower, somewhat irregular side to side displacements (dotted lines). Dotted lines represent wing positions 0.12 s after solid lines.

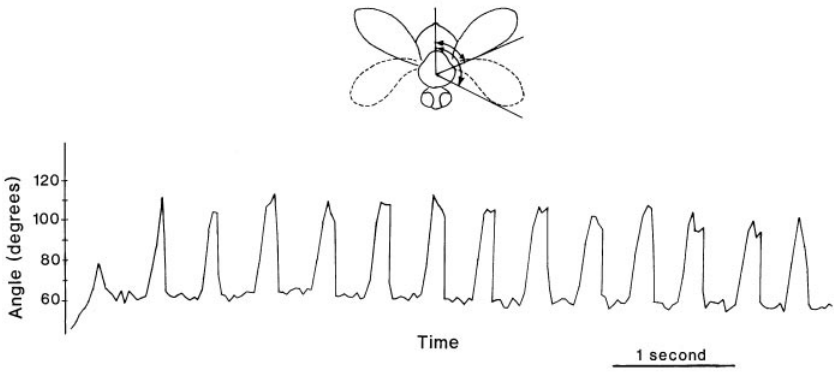

Figure 7. Wing buzzing involved rapid wing vibrations during which there were quick forward and backward movements. The graph shows approximate changes in wing positions over time (angle with longitudinal body axis; measurements were somewhat imprecise due to the blurred outlines of the wings, especially when the wings were moved anteriorly). Forward and backward movements were relatively rapid, and the wings were kept in the anterior position for shorter times than in the posterior position.

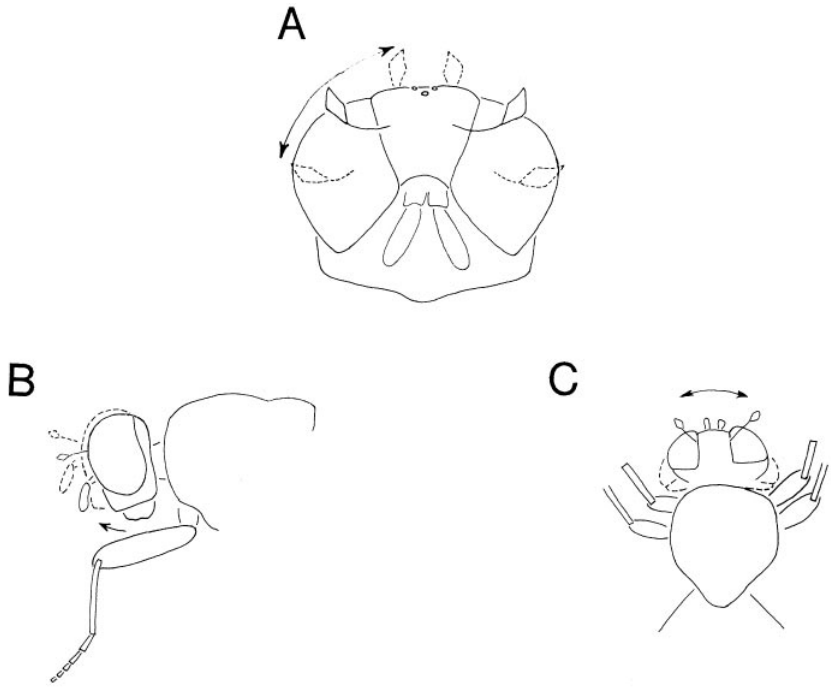

Figure 8. Ranges of movement of the male's head during head rocking. The strongest movements were rotatory (A), but dorso-ventral (B) and lateral (C) deflections also occurred. B and C were drawn directly from video images; the displacements in A were extrapolated from changes in the positions of the male's capitate setae seen in lateral views.

Discussion

Our observations clarify some behavioral details on which there have been previous contradictory reports. Zapién et al. (1983) indicated that when the male lept toward a female, he "jumped over the head of the female, turned and mounted her." The videos showed, however, that not only did the male consistently land on the female more or less facing the posterior end of her body and then turn 180° as described by Feron (1962), but he also rocked his body in a distinctive manner before making the turn.

Arita & Kaneshiro (1986) reported that the rectal epithelium is extended during the entire courtship sequence prior to mounting. At least in the strains we observed, the epithelium was everted during wing vibration, but was retracted during wing buzzing. Arita & Kaneshiro (1985) also mention that the male approaches the female "until he is almost touching the female's antennae with his second pair of fronto-orbital bristles." The flies of this study also approached females closely before leaping, but in at least some cases it was certain that they did not touch them with these bristles (e.g. Fig. 4) (the female's arista was generally not visible in the images, but some mounting attempts were launched from distances larger than the length of the arista;) (V. Mendéz, Universidad de Costa Rica, Ciudad Universitaria, Costa Rica, for

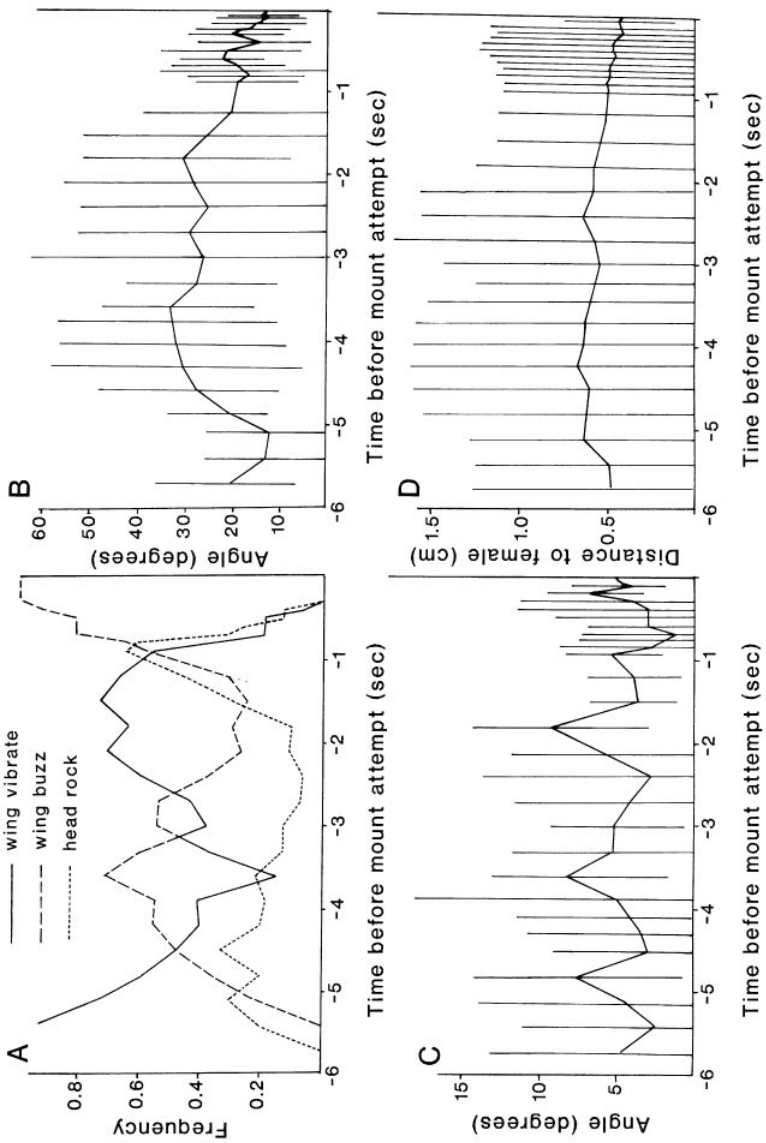

Figure 9. Changes in several variables during the last 6 sec prior to mounting attempts (B-female orientation; C-male orientation) (N=17).

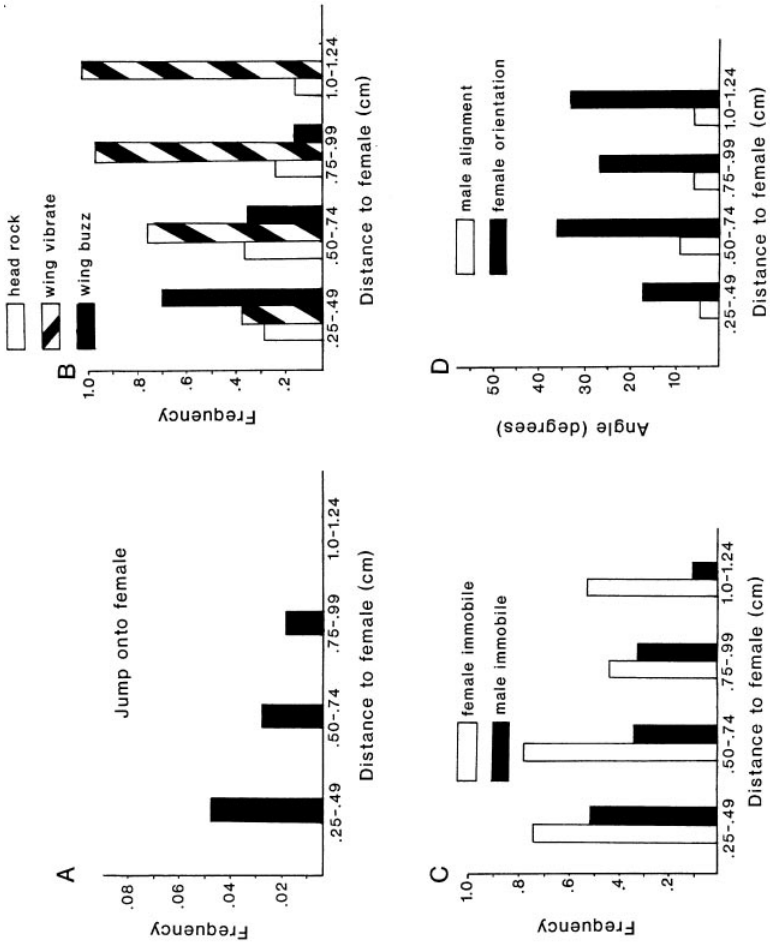

Figure 10. Changes in several variables as a function of the distance between male and female.

TABLE 1. AVERAGE VALUES (± 1 STANDARD DEVIATION) OF VARIABLES ASSOCIATED WITH MALE BEHAVIOR PATTERNS IN A RANDOM SAMPLE OF MALES. DURATIONS ARE IN SECONDS AND IN MALES JUST PRIOR TO LEAPING ONTO A FEMALE. DATA FROM MALES OF THE 14-YEAR AND THE 4.5-YEAR STRAINS ARE COMBINED.

	Random Sample	N	Behavior Prior to Leaping onto a Female	N
Reorient when female moves (sec)	0.82±0.83	212	0.84±0.76	50
Wing vibration	7.44±5.12	79	2.48±3.48	50
Wing buzz	3.33±3.31	34	3.63±3.17	50
Head rock (sec)	4.65±4.72	7	0.83±0.75	17
Distance prior to jump (cm)			0.42±0.80	29

further data on this point) (in contrast, both bristles and antennae touched in some aggressive head-to-head interactions between males—RDB & WGE, unpublished data). This suggests that the bristles may form part of the visual stimuli provided by head rocking (Arita 1983 cited in Arita & Kaneshiro 1986), and a positive effect on possible male mating success has been confirmed by experimental removal of the bristles (V. Mendéz).

The detailed analysis of male behavior just before mounting attempts also revealed new aspects of courtship not previously described. As previously reported, males buzzed their wings and rocked their heads prior to leaping onto the female, but head rocking was intermittent, and ceased during the 0.3 s immediately preceding the leap. Perhaps the male was better able to orient his leap by holding his head still just before leaping. The decision to mount the female may thus occur several tenths of a second before the leap itself.

The courtship song recorded by Webb et al. (1983) and signal II of Rolli (1976) probably correspond to wing buzzing. The frequency of pulses of sound (1 per 0.18 s - Webb et al. 1983) was similar to the frequency of forward wing displacements observed in the videos (1 per 0.26 s). Feron (1962) mentions a slightly slower frequency of 1 per 0.5 s, and notes that the female's wings were apparently slightly raised by the air currents produced by the male's wing movements. Since the rectal epithelium is retracted during wing buzzing, this behavior presumably represents an acoustic (or mechanical?) rather than a chemical stimulus.

The male's distance to the female was smaller, he was oriented more directly toward her, and she was oriented more directly toward him when a male attempted to mount the female than when he abandoned courtship, suggesting that these variables may be involved in triggering mounting attempts. None of these variables showed clear changes in the few seconds preceding mounting attempts, however, so they are probably not immediate triggers of mounting.

Using male behavior to tentatively deduce which possible stimuli are important, it appears that there are three phases of male courtship in medflies: a preliminary phase (initial attraction; wing vibration) in which odors play an important role; a subsequent close-range phase (head rocking, wing buzzing) in which visual and vibratory stimuli are important; and a final contact phase (body rocking, rubbing the female's abdomen and lifting her oviscape, nipping with genitalic surstyli) in which mechanical stimuli dominate. Further observations are needed to test these ideas and the possibility that (1) the behavior of mass-reared and wild flies is different and (2) the flies

facultatively modify their behavior when conditions, such as crowding, are changed (RDB & WGE, unpublished data).

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