Journal of Insect Behavior, Vol. 17, No. 2, March 2004 (© 2004)

Mating Behavior of *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae) and the Effect of Female Mating Frequency on Offspring Production

Ling-lan Cheng,[1] Ralph W. Howard,[2] James F. Campbell,[2]
Ralph E. Charlton,[1] James R. Nechols,[1] and Sonny B. Ramaswamy[1,3]

Accepted September 18, 2003; revised December 16, 2003

The courtship behavior of Cephalonomia tarsalis, *a solitary semiectoparasitoid of* Oryzaephilus surinamensis, *was investigated in the laboratory. Courtship behavior includes a series of stereotypic movements. Males play the most active role, executing the majority of courtship action, and females respond with relatively limited observable behaviors. Males typically keep antennae still during encounters with females prior to mounting, which may be correlated with recognition of the female's sexual status. After mounting, males display a series of movements on females, such as antennae touching female's antennae, antennae or mouth touching female's head or thorax, and walking around on female, which may serve to stimulate females towards increased receptivity. Females signal receptivity by assuming a stereotypical posture of remaining stationary, with head down, and antennae still in front of the body. The male then inserts his aedeagus and the pair copulates. After an average of 40.4 s of copulation, females signal the end of copulation by waving the antennae and moving away from the copulation site. Males continue copulating for a short time after females start moving but dismount soon thereafter. After dismounting, the two wasps move away from each other immediately, and they typically begin grooming. Neither males nor females exhibit mating preference based on mate's mating status in both choice and no-choice tests.*

[1] Department of Entomology, Kansas State University, Manhattan, Kansas 66506.
[2] Biological Research Unit, Grain Marketing & Production Research Center, 1515 College Avenue, Manhattan, Kansas 66502.
[3] To whom correspondence should be addressed. Fax: (785) 532-6232. e-mail: sonny@ksu.edu.

0892-7553/04/0300-0227/0 © 2004 Plenum Publishing Corporation

The male is polygynous and the mated female can mate multiple times within the first 3 days after starting oviposition. However, female mating frequency does not affect the production of female progeny.

KEY WORDS: saw-toothed grain beetle; parasitoids; courtship; mating preference; mating frequency; biological control.

INTRODUCTION

The saw-toothed grain beetle, *Oryzaephilus surinamensis*, is a cosmopolitan stored-product pest. Both larvae and adults cause damage. It is a secondary pest on grain damaged by other insects, such as the grain weevil, *Sitophilus granarius*, and also a pest of processed grain (Jefferies, 1966). Contact insecticides and fumigants are the primary means used for controlling this pest. However, due to development of resistance to major insecticides (Muggleton, 1987; Herron, 1990; Muggleton *et al.*, 1991; Collins *et al.*, 1993), high mammalian toxicity of phosphine (Willers, 1999), and a pending ban of methyl bromide (Anonymous, 1995), there is a need to develop more of an integrated pest management approach.

One potential component of integrated management of stored product pests is the use of natural enemies as biological control agents. This approach has many advantages over traditional chemical control (Schöller and Flinn, 2000). Natural enemies leave no harmful chemical residues. They are self-targeting, saving the grain manager from having to treat an entire grain mass. They can be removed easily from bulk grain using normal cleaning procedures before milling. The environmental conditions associated with stored products are generally favorable for natural enemies, and storage structures reduce beneficial insect emigration. To date, 58 species of predators and parasitoids of 79 stored-product pests have been studied experimentally (Schöller, 1998). In addition, there have been several practical uses of natural enemies in the control of stored-product pests, e.g., *Teretriosoma nigrescens* against *Prostephanus truncatus* (Pöschko, 1993; Boye *et al.*, 1994), *Trichogramma evanescens* and *Habrobracon hebetor* against stored-product moths (Cline *et al.*, 1984; Keever *et al.*, 1986; Prozell *et al.*, 1996), and *Cheyletus eruditus* against *Acarus siro*, *Tyrophagus putrescentiae*, *Lepidoglyphus destructor*, and *Glycyphagus domesticus* (Solomon, 1967; Berger, 1991).

Cephalonomia tarsalis can parasitize either larvae or pupae of the saw-toothed grain beetle; but the larvae are more commonly attacked (Ashmead 1893). Its basic biology has been reported by Powell (1938). Female wasps first sting and paralyze the host larva and then deposit eggs. Reproduction in *C. tarsalis* involves a system of haplodiploid sex determination. Virgin females produce only male offspring, while mated females can produce both males and female offspring. The eggs are deposited either singly or in pairs on the thoracic segments of the host. If eggs are deposited in pairs by a mated female, they will always be one female and one male egg. The eggs hatch within 24 h after oviposition. The larva develops as a semiectoparasitoid. That is, it feeds internally, but only the head and prothoracic segment are inside of the host while the remainder of the body is outside. The host is consumed in about 4 days. The larva then spins a cocoon in which it pupates. The pupal stage lasts about 8–9 days. Male wasps emerge about 1–2 days prior to the females. Powell (1938) indicated that in cases where male and female pairs were reared together, the male upon emergence from the cocoon would enter the female cocoon and copulate with her inside the cocoon. Males live for about 6 days and may copulate with many different females. The females live for about 35 days.

Howard *et al.* (1998) reported the host-finding, -recognition, and -acceptance behavior of *C. tarsalis*. They suggested that the parasitoid locates and recognizes the host beetle mainly through cuticular chemical cues perceived by antennae as well as host movement once contacted. Vision played only a limited role in host finding and recognition. They also found that the parasitoids will paralyze and hide several larvae before commencing oviposition and suggested that such behavior would increase the potential efficacy of this parasitoid as a biological control agent since paralyzed beetle larvae never recover.

Howard (1998) indicated that male and female *C. tarsalis* have the same cuticular hydrocarbons but differ in absolute quantity and in relative proportions. These hydrocarbon profiles are dynamic and respond to ontogenetic, reproductive, and nutritional effects. Males can distinguish female cocoons from male cocoons, spending longer periods of time walking on female cocoons and rapidly antennating them. On the other hand, when artificial cocoons treated with polar or hydrocarbon fractions of female–cocoon–hexane extracts were presented, males spent more time on polar fraction-treated cocoons. Males also spent more time on artificial cocoons treated with volatiles emanating from females plus female hydrocarbons than on cocoons treated with female volatiles plus male hydrocarbons. Howard (1998) therefore suggested that females may release a volatile sex pheromone but that the hydrocarbons may serve as a secondary gender recognition cue.

While some studies have been conducted on the host-searching behavior and chemical biology of this parasitoid, little is known of its mating behavior. Studies of insect mating behavior help in the precise identification of a species (van den Assem and Povel, 1973; Matthews, 1975), the evaluation as biological control agents, and the design of mass-rearing programs. This study was conducted to provide a quantitative description of the courtship behavior of *C. tarsalis*, as well as to test for a possible mating preference and the effect of female mating frequency on offspring production.

MATERIALS AND METHODS

Insects

The parasitic wasp, *Cephalonomia tarsalis*, and its host, *Oryzaephilus surinamensis*, were collected from farm-stored wheat in Kansas and have been in laboratory culture since 1994. *Oryzaephilus surinamensis* was reared on rolled oats with 3% brewer's yeast. *Cephalonomia tarsalis* was reared on fourth instar larvae of *O. surinamensis* in wheat. Both the wasp and the host cultures were held in a rearing chamber at 30 ± 1°C and 55 ± 10% RH with a photoperiod of 16:8 (L:D) h. Here, and throughout this paper, mean data are presented as mean ± standard error of the mean unless stated otherwise. All experimental wasps were less than 24 h old and were held in individual vials after pupation to avoid contact with other wasps prior to experiments. Unless otherwise noted, the male wasps used in tests were produced by virgin females. All experiments were carried out between 0800 and 1700. During this period, this wasp readily exhibited mating activities.

Courtship Sequence

One female and one male wasp were anesthetized on ice for 30 s. The female was introduced first into an observation cage, followed by the male. The observation cage was constructed by affixing a piece of foam 2 mm thick on a glass microscope slide with double-sticky tape. A 7 × 4-mm cell was cut in the center of the foam, and a piece of light blue paper was placed under the foam to provide contrast for video recording and as a purchase for the insects. After introduction of the wasps, a cover slide was used to cover the cage top to prevent escape of wasps. The courtship behavior, from initial interaction between the two wasps to postcopulation behavior, was recorded using a Panasonic digital Hi-8 video camera, Model WV-CP4100 (Panasonic Broadcast and Television Systems Co., Secaucus, NJ), attached to a Wild M8 stereomicroscope (Wild Heerbrugg Ltd., Heerbrugg, Switzerland) and connected to a Sony digital Hi-8 video cassette recorder, Model EV-S7000 (Sony Corporation, Park Ridge, NJ). Lighting was provided by a fiber optic microscope continuous ring light and shielded by a filter which was put on top of the observation cage above the cover slide to give an intensity of 2500 lux (±10%) at the level of the insects. Twenty-eight individual matings between nonsibs were recorded.

Detailed behavioral categories and related timing were determined through slow-motion video analysis (one-fifth normal speed), and recorded with Noldus Observer version 3.0 behavioral analysis software (Noldus Information Technology b.v., Wageningen, The Netherlands). Frequencies associated with transitions from one behavior to another were tabulated and translated into a first-order transition probability matrix using established methods (Fagen and Young, 1978), with the modification of Charlton and Cardé (1990), which provide equal weighting to the individual behaviors in the consolidated matrix. Self-transitions and impossible transitions were left as blanks. These probabilities were then used to generate an ethogram of courtship behavior.

Mating Preference

No-Choice Tests

Pairs of virgin female (VF) and virgin male (VM), virgin female and mated male (MM), mated female (MF) and virgin male, mated female and mated male, and female cocoon and virgin male (both cocoon and virgin male were obtained from the same pair of eggs laid by a mated female) were held in 1.8 × 1.5-cm observation cages. The observation cages used in this experiment were larger than those described above but were constructed in the same manner. Total time spent by the male to mount, court, and copulate with the female in each pair was recorded. Different observation cages were used for the different combinations to avoid possible pheromone contamination. The wasps were anesthetized on ice for 30 s before introduction into cages. Female wasps were always allowed to recover before the males. Observations were made at 23 ± 2°C for a period of 15 min under room fluorescent lighting. Female cocoons used in this test were 7 days old, at which time pharate adult development was complete. Mated wasps had copulated once 2 h before the tests with virgin mates that were less than 24 h old. These observations were repeated four times for a total of 55 replications for each combination (three times with 15 replications and one time with 10 replications) were carried out; but only 17 replications were used for the combination of female cocoon × virgin male. Data were subjected to life table (times) analysis (Statgraphics Plus 4.0, 1998) that analyzes the probability of failure of a specific behavior over time (survival analysis). Observations were censored if after 900 s the male did not mount the female. Censored pairs were excluded from the analysis of time spent courting and copulating. If the male mounted and courted the female more than once before successful copulation, the latency to first mounting and the time spent courting that resulted in copulation were used in the analysis.

Choice Tests

Virgin or mated males or virgin or mated females were confined with pairs of virgin and mated individuals of the opposite sex in the same type of observation cages as used in the no-choice tests. The mating status of individual to mate successfully first was recorded. Mated wasps had one previous copulation 1 h before the test with virgin mates that were less than 24 h old. To distinguish individuals with different mating status, two different colors of Crayola washable markers were used to mark wings. The colors used for each combination were alternated between replications. Wasps of the same sex were introduced into observation cages first, and wasps of the opposite sex then followed. Different cages were used for each combination. These observations were carried out under the same environmental condition as in the no-choice tests, and it was repeated three times with a total of 45 replications for each combination. Data were analyzed using a two-way contingency table and χ^2 analysis (Zar, 1984).

Effect of Female Mating Frequency on Offspring Production

The influence of the number of female copulations on offspring production was determined using the following treatments: female having (1) only one copulation within 24 h after emergence, (2) three copulations successively within 24 h after emergence, and (3) no copulations. Every copulation was with a nonsib virgin male emerging within 24 h. Wasps from each treatment were introduced individually into a 1.5-cm-high × 7.0-cm-diameter petri dish containing 12.09 ± 0.43 g of wheat and 40 14-day-old saw-toothed grain beetle larvae (i.e., ovipositional unit). A cotton ball wetted with 10% honey water was added to the dish. The wasps were transferred into new ovipositional units twice a week until wasp death. All ovipositional units were held at 30 ± 1°C, 55 ± 10% RH, and 16:8 (L:D)-h photoperiod and covered with a piece of white paper. The total number of pupae produced and the resulting male and female adults were recorded. Twenty replications were used for each treatment.

RESULTS

Courtship Sequence

The conditional probabilities and sequences associated with behavioral transitions of courtship behavior of *C. tarsalis* are illustrated in Fig. 1, and descriptions of the behaviors are given in Table I. Behavioral transitions with

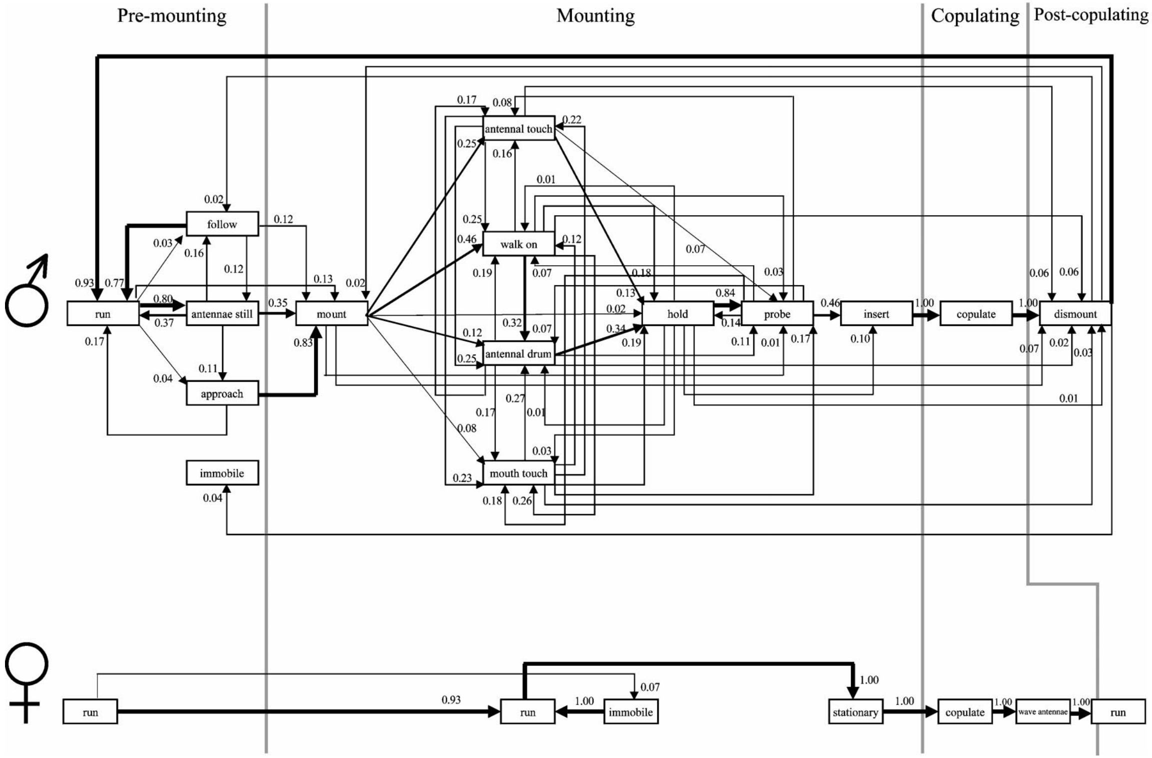

Fig. 1. Flow sequence in courtship behavior of *C. tarsalis* (based on 28 successful courtships). Thickness of black lines and associated decimal numbers denote the behavioral transitions and conditional probabilities (——, transition probabilities 0.00–0.10; —= 0.11–0.30, —, 0.31–0.60; ‾‾‾, 0.61–1.0). Transitions with probabilities less than 0.1 are not shown. Gray lines group corresponding behaviors in both sexes in various courtship phases.

Table I. Courtship Behavior of *C. tarsalis*

Behavior	Description
Male	
Follow	Running immediately behind running female
Antennae still	Stopping near female and keeping antennae immobile
Approach	Moving slowly toward female; may or may not keep antennae still
Mount	Getting on the female in a dorsal riding position beginning with the front legs climbing on from anterior, posterior, or sides of female
Antennal touch	Antennae contacting with female's antennae
Antennal drum	Antennae tapping female's head or thorax
Walk on	Moving around female's body but without any antennal or mouth contact
Mouth touch	Mouth contacting with female's head or thorax
Hold	Grasping female's posterior abdomen with middle and hind legs
Probe	Extending aedeagus to touch female's genital orifice (abdominal tip) while doing walk on and/or antennal drum and/or mouth touch
Insert	Putting aedeagus into female's genital orifice with or without holding the female's posterior abdomen
Copulate	Copulating with female by assuming posterior dorsal or side position with middle and hind legs holding female's rear end of abdomen; vibrating antennae; rhythmically contracting body, forelegs sweeping on the female's dorsal thorax or abdomen and head nodding on, as well as mouth touching, female's dorsal body
Dismount	Getting off the female
Female	
Stationary	Keeping body immobile, head down, and antennae still in the front of body
Copulate	Copulating with male while remaining stationary and antennae lowered down
Wave antennae	Vibrating antennae after copulating for a period of time
Male & female	
Run	Moving rapidly around the arena
Immobile	Staying still in the arena

probabilities less than 1% are not shown. When a female and a male wasp were released into the cage, both initially ran around the arena. During this period, males typically exhibited "antennae still" when they encountered females (80% of transitions). However, females always ran away immediately after they encountered males. If a male did not mount the female after "antennae still," he would run around the arena until encountering the female again. Some males went directly from running to mounting a female without any premounting behaviors (13% of transition). Males mounted from the female's anterior (10 of 38), sides (8 of 38), or posterior (20 of 38) but, once mounted, quickly reoriented to face the female's anterior end.

The mean latency (amount of time elapsing before a behavior occurred) to mounting was 23.3 s (range: 1.3–77.2 s).

After mounting, males exhibited a series of movements on the female's body including "antennal touch," "antennal drum," "mouth touch," and "walk on." Not all males displayed all of these movements, but 24 of 28 males displayed at least three of the movements and switched frequently among them. While walking around on the female, 18 of 28 males changed orientation but always returned to facing anteriorly before initiating probing.

Following a period of movement on the female's body, the male typically used his middle and hind legs to hold the female's posterior abdomen and probed her abdominal tip with his aedeagus, but males could also probe without holding (35.7%). Females continued to move around the arena during all of the male's actions. At a certain point, females stopped running and remained stationary with their heads down and antennae still, and males inserted their aedeagus into the female's genital orifice. The mean latency from the time female was mounted until she became stationary was 20.2 s (range: 2.3–66.9 s). The mean and cummulative durations of each type of behavioral bout during courtship were as follows: antennal touch, 0.55 ± 0.05 and 38.6 s; antennal drum, 0.55 ± 0.04 and 41.5 s; mouth touch, 0.57 ± 0.05 and 50.4 s; walk on, 14.77 ± 1.81 and 121.7 s, and "probe," 3.10 ± 0.35 and 272.4 s.

In copula, 26 of 28 males assumed posterior dorsal position, while only 2 of 28 assumed a side position. All males faced in the same direction as females. After a period of copulation, the female started to wave her antennae, then moved away from the copulation site. The mean latencies for females from copulation to waving antennae and from waving antennae to moving away were 21.46 s (range: 1.9–42.7 s) and 9.18 s (range: 2.0–26.8 s), respectively. The male initially continued to copulate but eventually dismounted. The mean latency from a female moving away to a male dismounting was 12.71 s (range: 2.7–35.7 s). Copulation lasted an average of 40.4 s (range: 32.4–52.0 s). Following dismount, in 93% of observations both individuals again ran around the arena and groomed themselves by preening antennae with front tibia and/or rubbing the abdomen with hind tibia.

Mating Preference

No-Choice Tests

None of the males copulated with females inside cocoons, and 15 of 17 males did not even touch the cocoons. The biased (due to censored observations) mean latencies to mounting were 91.4 ± 11.4 s for virgin males with virgin females, 265.4 ± 38.2 s for mated males with virgin females, 138.7 ± 15.6 s for virgin males with mated females, and 273.6 ± 35.9 s for mated males with mated females. Latencies for both virgin and mated males to mount mated females were significantly longer than that to mount virgin females. Latency for mounting virgin females was significantly longer for mated males than for virgin males (Fig. 2). However, once mounting

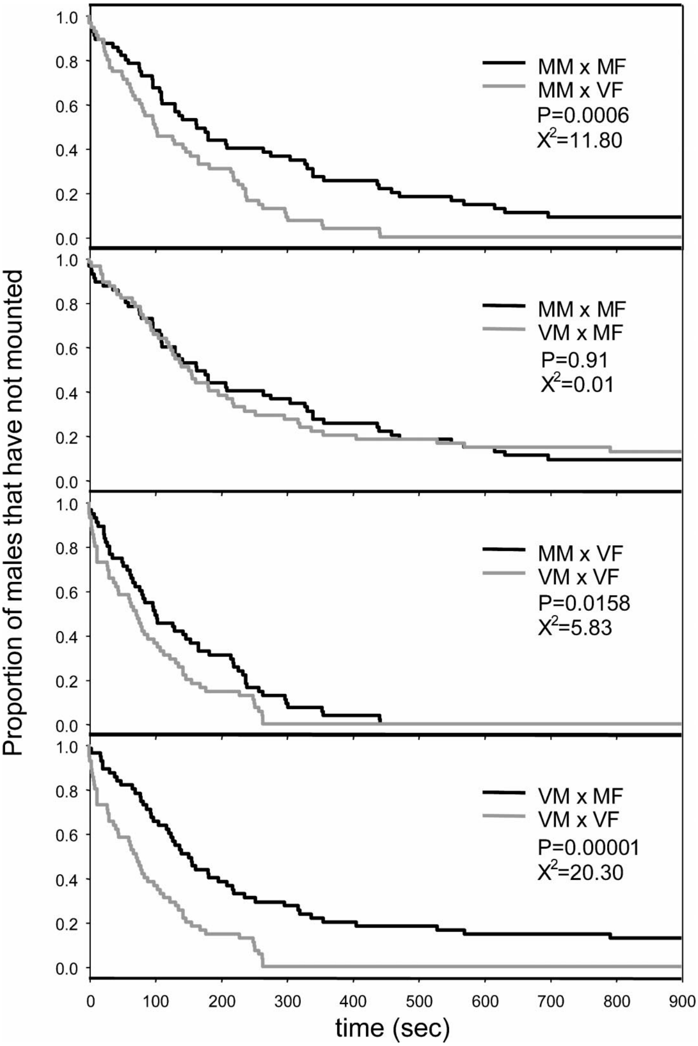

Fig. 2. Proportion of males that have not mounted as a function of time after initial exposure. *P* values indicate differences between treatments based on logrank test ($n = 55$) (Statgraphics Plus 4.0, 1998).

had occurred, the time spent by males for courting and copulating was not significantly different among the various combinations.

Choice Tests

The ratios of virgin to mated wasps that successfully copulated with mates in various choice combinations are as follows: 22/23, $\chi^2 = 0.011$, $P > 0.9$, df = 1, for the combination of VM+VF/MF; 28/17, $\chi^2 = 0.914$, $P > 0.1$, df = 1, for the combination of MM+VF/MF; 25/20, $\chi^2 = 0.100$, $P > 0.1$, df = 1, for the combination of VF+VM/MM; and 29/16, $\chi^2 = 1.373$, $P > 0.1$, df = 1, for the combination of MF+VM/MM. There were no significant differences in all combinations of giving mate choice to males and females of different mating status. Besides, when one virgin and one mated female were together presented to either a virgin or a mated male, the latency to mounting was not significantly different between both males of different mating status ($P = 0.903$, $H = 0.015$, df = 1; Kruskal–Wallis test).

Effect of Female Mating Frequency on Offspring Production

There was no significant difference in total offspring production among the three groups of females with different number of matings ($P = 0.55$, $H = 1.21$, df = 2; Kruskal–Wallis test). Between the female groups with one mating and three matings, there was no difference in the proportion of female progeny ($P = 1.19$, $H = 41.10$, df = 1. However, females that mated multiple times lived for significantly shorter times than did virgin females ($P = 0.04$, $H = 6.23$, df = 2) (Table II). In all treatments, total offspring production and proportion of female progeny declined after week 3 (Fig. 3). Offspring production then gradually reached 0 at week 7 in both one- and three-mating treatments. Although after week 7, the female group with no mating could still produce offspring, only 3% of the total offspring were produced during this period. Over 90% of females in the groups with one and three matings continued to produce offspring until they died, but over 98% of female offspring were produced before week 5. Females in the no-mating treatment lived for 1.5 to 10 weeks after cessation of oviposition.

DISCUSSION

Male *C. tarsalis* play the most active role in courtship, executing the majority of observable behaviors, and females apparently are passive with limited visible response. During premounting, males typically vibrate their

Table II. Fecundity and Longevity of *C. tarsalis* Females with Various Numbers of Matings

	No. of matings		
	0	1	3
No. of cocoons produced/female	179.3 ± 10.00 a	193.3 ± 8.20 a	177.5 ± 10.80 a
Proportion female offspring	0.0 b	0.5 ± 0.02 a	0.5 ± 0.03 a
Longevity (days)	50.5 ± 4.50 a	40.6 ± 1.60 ab	37.3 ± 1.90 b

Note. Means followed by different letters within the same row are significantly different at $P \leq 0.05$ (Kruskal–Wallis test).

antennae while running around the arena but keep their antennae still when they encounter females. Males likely perceive some chemical cue from the female to recognize her sexual status. Further support for the role of chemical cues is that males do not respond to a mated female that has been ovipositing

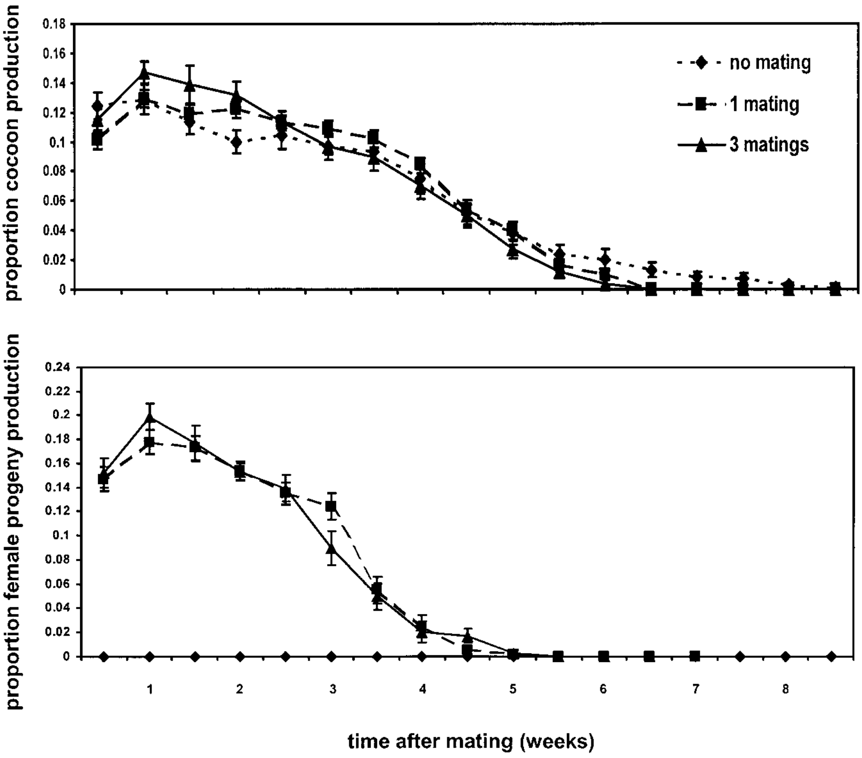

Fig. 3. Mean proportion of cocoon and female progeny (±SE) produced over time by females with various numbers of matings.

for more than 3 days, but they will extend the aedeagus to probe anesthetized or freshly dead females, although without many of the other courtship behaviors (Cheng, personal observation). Many parasitic Hymenoptera produce sex pheromones (Vinson, 1972; Eller *et al.*, 1984). These sex pheromones often comprise two or more components, one component serving for long range attraction of males by females (Reed *et al.*, 1994) and the others mediating subsequent courtship behavior, and can be produced by both males and females. Howard (1998) showed that volatiles emanating from female *C. tarsalis* are involved in mate recognition in males, and cuticular hydrocarbons may serve as a secondary gender recognition cue. How chemical cues are involved in the courtship behavior of *C. tarsalis* deserves further study.

After mounting, males display a series of movements on the females' body. Movements such as "antennal touch," "antennal drum," and "mouth touch" involve contact with the female's anterior region and appear to stimulate the female, thereby allowing them to become receptive. The fact that males stroke female antennae with their own after mounting has been observed in several other parasitic Hymenoptera (Barrass, 1960, 1976; van den Assem, 1996; Isidoro *et al.*, 1996; Bin *et al.*, 1999; Ruther *et al.* 2000). It has been suggested that males transfer sex pheromones to the female's antennae during stroking (Isidoro and Bin, 1995; Isidoro *et al.*, 1996). Furthermore, males of *N. vitripennis* have been shown to release a chemical from their mouthparts during antennal stroking to stimulate receptiveness in females (van den Assem *et al.*, 1980).

Researchers earlier considered that female parasitoid wasps are usually passive and not sexually selective in mating (van den Assem, 1986). In our observations, female *C. tarsalis* always ran away from males, but most of them eventually became receptive after a period of male courting. Successful copulation always coincided with the female remaining stationary, with head down and antennae still. In a few cases, female *C. tarsalis* appeared to reject males by not remaining stationary and lowering the head and kicking at males, even after male courtships of more than 20 min. After a period of copulation, females wave their antennae and move away from the copulation site, likely signaling completion. If males do not dismount within a short period of time after females become active, females will use the middle or hind legs to kick at male. All of these behaviors indicate that female *C. tarsalis* are selective to some degree. Changes in female posture when receptive also have been demonstrated in other parasitoids (Barrass, 1960; van den Assem, 1974; Orr and Borden, 1983; Field and Keller, 1993a).

The duration of copulation is brief in *C. tarsalis*. Short copulations have been reported for most parasitic wasps, typically lasting only 10–20 s and seldom lasting for more than a minute or two (van den Assem, 1986). Short courtship and copulation periods may be selectively advantageous to parasitoids. The longer a pair remains *in copula*, the greater the risk of exposure to enemies or to unmated male rivals. Although postcopulatory guarding has been observed in parasitic wasp species (Gordh and Debach, 1978; Kajita, 1986; Field and Keller, 1993b; Allen *et al.*, 1994; Assem and Werren, 1994; Eberhard, 1994; Ruther *et al.*, 2000), postcopulatory guarding was not observed in *C. tarsalis*.

Eberhard (1994) stated that copulatory courtship is common in many insect species and might function in inducing the female to remain still, thus increasing the male's chances of fathering the offspring. *Cephalonomia tarsalis* males exhibit several behaviors while copulating (i.e., vibrating antennae, rhythmical contraction of body, forelegs sweeping on female's dorsal thorax or abdomen, and head nodding on, as well as mouth touching, the dorsal part of the female's body) that may be considered copulatory courtship behaviors.

The results from both no-choice and choice tests implied that males could distinguish virgin females from mated females, and they responded more quickly to virgin females than to mated ones in terms of latency to mounting. However, males did not show significant preference for virgin females under choice situation. This might be due in part to the male being unable to distinguish the female's mating status when the two females are in close proximity (e.g., if pheromone cues are used). Although virgin males attempted more vigorously than did mated males in mounting virgin females under no-choice condition, they did not show significant dominance in mating with either virgin or mated mates under choice condition. In part, this may be due to the fact that the response of a mated male to a virgin female increased when a virgin male was nearby.

Most parasitic wasps are not reported to exhibit mate choice except in a few cases; e.g., *Habrobracon hebetor* females favor their own sons over less closely related males (Petters *et al.*, 1985), larger males of *Megalothynnus klugii* and *Macrothynnus* sp. are more likely to get mates, and *M. klugi* males prefer larger females (Alcock and Gwynne, 1987). For *C. tarsalis*, we did not observe any difference in mating success based on mating status (reported in this paper) or male body size (Cheng *et al.*, 2003). However, there may be other factors that under natural conditions lead to selection prior to female encounter. In addition, sperm competition after insemination and female's cryptic choice in using sperm from multiple mates may also be occurring. Further research in this area is needed to fully understand sexual selection in *C. tarsalis*.

Some bethylid males have been noted chewing into female cocoons and copulating (Gordh, 1976; Mertens, 1980; Remadevi *et al.*, 1981; Gordh *et al.*, 1983). In *C. tarsalis*, Powell (1938) reported the same, and Howard (1998) showed that males could distinguish female cocoons from male cocoons spending longer period of time walking on female cocoons and rapidly antennating them. However, this phenomenon was not found in the current study. At this time the reasons for this difference are unknown. Some possible reasons include the degree of genetic relatedness between the females and the males used, strain differences, or differences in the experimental protocol.

In *C. tarsalis*, mated females, after initiating oviposition, mated again during the first 3 days (Cheng, personal observation), but after 3 days, they would not mate again, even after they stopped producing female progeny. In some other parasitoid species, sperm-depleted females can mate again; e.g., *Nasonia vitripennis*, *Habrobracon lineatellae*, *Melittobia acasta*, *Goniozus gallicola*, *Trichogramma minutum* (Ridley, 1988). Virgin and mated female *C. tarsalis* did not differ in total progeny production, but several studies with other parasitoid species have indicated that virgin females attack fewer hosts than do mated females (Avidov *et al.*, 1967; Donaldson and Walter, 1984; Tagawa, 1987; Antolin, 1989; Michaud, 1994). For *C. tarsalis*, multiple mating did not increase the proportion of female offspring and this has also been demonstrated with some other parasitoid species; e.g., *Monodontomerus obscures*, *Diadroma*, *Aphytis* (Ridley, 1988). Presence of males can reduce fecundity and the proportion of female progeny (e.g., *Trichomalopsis apanteloctena* [Singh, 1998]) and might be a reason for mated, ovipositing females of *C. tarsalis* not to signal males that they are receptive after 3 days.

Many parasitic wasps produce offspring with different sex ratios at different periods of their lives. In some cases, fertilized females produce significantly more males soon after mating, and the proportion of females increases gradually thereafter (Donaldson and Walter, 1984). However, in the majority of species, females lay a high proportion of fertilized eggs immediately after mating (King, 1987; Brodeur and McNeil, 1994; Tillman, 1994). In *C. tarsalis*, females, regardless of mating once or three times, produced a higher proportion of female offspring immediately after mating. Subsequently, the proportion decreased gradually after 1.5 weeks and became male-biased after 3 weeks. This shift may be related to gradual depletion of sperm over time.

Ridley (1993) reviewed 97 species of parasitic Hymenoptera and found that solitary species (a female lays one egg on a host) tend to be monandrous, whereas gregarious species (a female lays several eggs in a host) tend to be polyandrous. He suggested that sibmating, which is common among gregarious species, might select for multiple mating, thus diversifying her progeny through mating with multiple males. *Cephalonomia tarsalis* is a solitary species, but we found that it mated multiple times. It is not clear under natural situations how often females are likely to encounter multiple males during her receptive period. In our study, immediately after the completion of copulation, the male and female quickly moved away from each other and, in natural situations, may be unlikely to reencounter each other. Further research on the spatial distribution of these parasitoids and the proportion of nonmated females in natural populations and mating studies conducted under more natural conditions are needed.

ACKNOWLEDGMENTS

We thank P. I. Nelson for assistance in statistical analyses and R. Speirs for rearing the insects. We also thank A. B. Broce, D. Stanley, P. A. Edde, and two anonymous reviewers for critically reviewing the manuscript. Voucher specimens (No. 137) were deposited in the Museum of Entomological and Prairie Arthropod Research, Department of Entomology, Kansas State University. This is Contribution No. 03-113-J of the Kansas Agricultural Experiment Station, Kansas State University, Manhattan.

REFERENCES

Alcock, J., and Gwynne, D. T. (1987). Courtship feeding and mate choice in thynnine wasps (Hymenoptera: Tiphiidae). *Aust. J. Zool.* **35:** 39–47.

Allen, G. R., Kazmer, D. J., and Luck, R. F. (1994). Postcopulatory male behavior, sperm precedence and multiple mating in a solitary parasitoid wasp. *Anim. Behav.* **48:** 635–644.

Anonymous (1995). Regulatory status of methyl bromide and priority review of methyl bromide alternatives. Pesticide Regulation (PT) Notice 95-4, Environmental Protection Agency, Washington, DC, July 13, pp. 1–4.

Antolin, M. F. (1989). Genetic considerations in the study of attack behaviour of parasitoids, with references to *Muscidifurax raptor* (Hymenoptera: Pteromalidae). *Fla. Entomol.* **72:** 15–32.

Ashmead, W. H. (1893). A monograph of the North American Proctotrypidae. *U.S. Natl. Mus. Bull.* **45**.

Avidov, Z., Rössler, Y., and Rosen, D. (1967). Studies on an Israel strain of *Anagyrus pseudococci* (Girault) (Hym., Encyrtidae). II. Some biological aspects. *Entomophaga* **12:** 111–118.

Barrass, R. (1960). The courtship behavior of *Mormoniella vitripennis* Walk. (Hymenoptera, Pteromalidae). *Behavior* **15:** 185–209.

Barrass, R. (1976). Inhibitory effects of courtship in the wasp *Mormoniella vitripennis* and a new interpretation of the biological significance of courtship in insects. *Physiol. Entomol.* **1:** 229–234.

Berger, H. K. (1991). Nützlingseinsatz auch im Vorratsschutz. *Der Pflanzenarzt.* **6:** 20–22.

Bin, F., Wackers, F., Romani, R., and Isidoro, N. (1999) Tyloids in *Pimpla turionellae* (L.) are release structures of male antennal glands involved in courtship behaviour (Hymenoptera: Ichneumonidae). *Int. J. Insect Morphol. Embryol.* **28:** 61–68.

Boye, J., Biliwa, A., Fischer, H. U., Helbig, J., and Richter, J. (1994). The dispersion pattern of *Teretriosoma nigrescens* Lewis (Col., Histeridae) after its release and monitoring of the occurrence of its host *Prostephanus truncatus* (Horn) (Col., Bostrichidae) in the natural environment in Togo. In Highley, E., Wright, E. J., Banks, H. J., and Champ, B. R. (eds.), *Proc. 6th Int. Working. Conf. Stored-Prod. Prot.*, CAB International, Wallingford, UK, pp. 1098–1102.

Brodeur, J., and McNeil, J. N. (1994). Life-history of the aphid hyperparasitoid *Asaphes vulgaris* Walker (Pteromalidae)—Possible consequences on the efficacy of the primary parasitoid *Aphidius nigripes* Ashmead (Aphidiidae). *Can. Entomol.* **126:** 1493–1497.

Charlton, R. F., and Cardé, R. T. (1990). Behavioral interactions in the courtship of *Lymantria dispar* (Lepidoptera: Lymantriidae). *Ann. Entomol. Soc. Am.* **83:** 89–96.

Cheng, L. L., Howard, R. W., Campbell, J. F., Charlton, R. E., Nechols, J. R., and Ramaswamy, S. (2003). Behavioral interaction between males of *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae) competing for females. *J. Insect Behav.* **16:** 625–645.

Cline, L. D., Press, J. W., and Flaherty, B. R. (1984). Preventing the spread of the almond moth (Lepidoptera: Pyralidae) from infested food debris to adjacent uninfested packages using the parasite *Bracon hebetor* (Hymenoptera: Braconidae). *J. Econ. Entomol.* **77:** 331–333.

Collins, P. J., Lambkin, T. M., Bridgemen, B. W., and Pulvirenti, C. (1993). Resistance to grain-protectant insecticides in coleopterous pests of stored cereals in Queensland. *Aust. J. Econ. Entomol.* **86:** 239–245.

Donaldson, J. S., and Walter, G. H. (1984). Sex ratios of *Spalangia endius* (Hymenoptera: Pteromalidae), in relation to current theory. *Ecol. Entomol.* **9:** 395–402.

Eberhard, W. G. (1994). Evidence for widespread courtship during copulation in 131 species of insects and spiders, and implications for cryptic female choice. *Evolution* **48:** 711–733.

Eller, F. J., Bartelt, R. J., Jones, R. L., and Kulman, H. M. (1984). Ethyl(Z)-9-hexade-canoate a sex pheromone of *Syndipnus rubiginosus*, a sawfly parasitoid. *J. Chem. Ecol.* **10:** 291–300.

Fagen, R. M., and Young, D. Y. (1978). Temporal patterns of behavior: durations, intervals, latencies, and sequences. In Colgan, P. W. (ed.), *Quantitative Ethology*, Wiley, New York, pp. 79–114.

Field, S. A., and Keller, M. A. (1993a). Courtship and intersexual signaling in the parasitic wasp *Cotesia rubecula* (Hymenoptera: Braconidae). *J. Insect Behav.* **6:** 737–750.

Field, S. A., and Keller, M. A. (1993b). Alternative mating tactics and female mimicry as post-copulatory mate-guarding behavior in the parasitic wasp *Cotesia rubecula. Anim. Behav.* **46:** 1183–1189.

Gordh, G. (1976). *Goniozus gallicola* Fouts, a parasite of moth larvae, with notes on other bethylids (Hymenoptera: Bethylidae; Lepidoptera: Gelechiidae). *Tech. Bull. USDA* **1524:** 1–27.

Gordh, G., and Debach, P. (1978). Courtship behavior in the *Aphytis lingnanensis* group, its potential usefulness in taxonomy, and a review of sexual behavior in the parasitic Hymenoptera (Chalc.; Aphelinidae). *Hilgardia* **46:** 37–75.

Gordh, G., Wolley, J. B., and Medved, R. A., (1983). Biological studies on *Goniozus legneri* (Hymenoptera: Bethylidae) a primary external parasite of navel orangeworm *Amyelois transitilla* and pink bollworm *Pectinophora gossypiella* (Lepidoptera: Pyralidae, Gelechiidae). *Contrib. Am. Entomol. Inst.* **20:** 433–468.

Herron, G. A. (1990). Resistance to grain protectants and phosphine in coleopterous pests of grain stored on farms in New South Wales. *J. Aust. Entomol. Soc.* **29:** 183–189.

Howard, R. W. (1998). Ontogenetic, reproductive, and nutritional effects on the cuticular hydrocarbons of the host-specific ectoparasitoid *Cephalonomia tarsalis* (Hymenoptera: Bethylidae). *Ann. Entomol. Soc. Am.* **91:** 101–112.

Howard, R. W., Charlton, M., and Charlton R. E. (1998). Host-finding, host-recognition, and host-acceptance behavior of *Cephalonomia tarsalis* (Hymenoptera: Bethylidae). *Ann. Entomol. Soc. Am.* **91:** 879–889.

Isidoro, N., and Bin, F. (1995). Male antennal gland of *Amitus spiniferus* (Brethes) (Hymenoptera: Platygastridae), likely involved in courtship behavior. *Int. J. Insect Morphol. Embr.* **24:** 365–373.

Isidoro, N., Bin, F., Colazza, S., and Vinson, S. B. (1996). Antennal gustatory sensilla and glands in some parasitic Hymenoptera: A critical morpho-functional approach. *J. Hym. Res.* **5:** 206–239.

Jefferies, D. J. (1966) Susceptibility of the saw-toothed grain beetle *Oryzaephilus surinamensis* (L.). In Cornwell, P. B. (ed.), *The Entomology of Radiation Disinfestation of Grain*, Pergamon Press, London, pp. 119–130.

Kajita, H. (1986). Role of postcopulatory courtship in insemination of two aphelinid wasps (Hymenoptera: Aphelinidae). *Appl. Entomol. Zool.* **21**(3): 484–486.

Keever, D. W., Mullen, M. A., Press, J. W., and Arbogast, R. T. (1986). Augmentation of natural enemies for suppressing two major insect pests in stored farmers stock peanuts. *Environ. Entomol.* **15:** 767–770.

King, B. H. (1987). Offspring sex ratios in parasitoid wasps. *Q. Rev. Biol.* **62:** 367–396.

Matthews, R. W. (1975). Courtship in parasitic wasps. In Price, P. W. (ed.), *Evolutionary Strategies of Parasitic Insects and Mites*, Plenum Press, New York, pp. 66–86.

Mertens, J. W., (1980). Life history and behaviour of *Laelius pedatus*, a gregarious bethylid ectoparasite of *Anthrenus verbasci. Ann. Entomol. Soc. Am.* **73:** 688–693.

Michaud, J. P. (1994). Differences in foraging behaviour between virgin and mated aphid parasitoids (Hymenoptera: Aphidiidae). *Can. J. Zool.* **72:** 1597–1602.

Muggleton, J. (1987). Insecticide resistance in stored product beetles and its consequences for their control. *In Proceedings 1986 British Crop Protection Conference—Pests and Diseases*, BCPC Monograph No. 37, pp. 177–186.

Muggleton, J., Llewellin, J. A., and Prickett, A. J. (1991). Insecticide resistance in populations of *Oryzaephilus surinamensis* and *Cryptolestes ferrugineus* from grain stores in the United Kingdom. In Fleurat-Lessard, F., and Ducom, P. (eds.), *proceedings of the 5th International Working Conference on Stored-Product Protection*, *Vol. 2*, Bordeaux, France, pp. 1019–1028.

Orr, D. B., and Borden, J. H. (1983). Courtship and mating behavior of *Megastigmus pinus* (Hym., Torymidae). *J. Entomol. Soc. BC* **80:** 20–24.

Petters, R. M., Kendall, M. E., Taylor, R. A. J., and Mettus, R. V. (1985). Time required for mating and the degree of genetic relatedness in the parasitic wasp, *Bracon hebeto* Say (Hymenoptera: Braconidae). *Melsheimer Entomol. Ser.* **35:** 21–27.

Pöschko, M. (1993). Biologie und wirtsspezifität von Teretriosoma nigrescens Lewis (Coleoptera: Histeridae). Ph.D. dissertation, Technische University Berlin, Berlin, Germany.

Powell, D. (1938). The biology of *Cephalonomia tarsalis* (Ash.), a vespoid wasp (Bethylidae: Hymenoptera) parasitic on the sawtoothed grain beetle. *Ann. Entomol. Soc. Am.* **31:** 44–48.

Prozell, S., Schöllesr, M., Hassan, S. A., and Reichmuth, Ch. (1996). Release of *Trichogramma evanescens* as a component of an integrated pest management programme in organic food bakeries and stores (Hymenoptera: Trichogrammatidae). In *Proc. 20th Int. Congr. Entomol.*, Florence, Ital.

Reed, H. C., Tan, S., and Reed, D. K. (1994). Evidence for a sex attractant in *Aphidius colemani* Viereck with potential use in field studies. *Southwest. Entomol.* **19:** 273–278.

Remadevi, O. K., Mohamed, U. V. K., and Abdurahiman U. C. (1981). Some aspects of the biology of *Perisierola nephantidis*, a larval parasitoid of *Nephantis serinopa. Polskie Pismo Entomol.* **51:** 597–604.

Ridley, M. (1988). Mating frequency and fecundity in insects. *Biol. Rev.* **63:** 509–549.

Ridley, M. (1993). Clutch size and mating frequency in parasitic Hymenoptera. *Am. Nat.* **142:** 893–910.

Ruther, J., Homann, M., and Steidle, J. L. M. (2000). Female-derived sex pheromone mediates courtship behaviour in the parasitoid *Lariophagus distinguendus. Entomol. Exp. Appl.* **96:** 265–274.

Schöller, M. (1998). Biologische bekämpfung vorratschädlicher arthropoden mit räubern und parasitoiden-sammelbericht und bibliographie, In Reichmuth, Ch. (ed.), *100 Jahre pflanzenschutzforschung, Wichtige arbeitsschwerpunkte im vorratsschutz. mitteilungen aus der biologischen bundesanstalt für land-und forstwirtschaft*, Heft 342. Parey, Berlin, pp. 85–189.

Schöller, M., and Flinn, P. W. (2000). Parasites and predators. In Subramanyam, B., and Hagstrum, D. (eds.), *Alternatives to Pesticides in Stored-Product IPM*, Kluwer Academic, Boston/Dordrecht/London, pp. 229–272.

Singh, R. N. (1998). Influence of male parasitoid on the fecundity, longevity and sex-ratio of *Trichomalopsis apanteloctena* Crawford parasitising puparia of *Blepharipa zebina* (Walker). *J. Biol. Control* **12:** 51–54.

Solomon, M. E. (1967). Experiments on predatory-prey interactions of storage mites. *Acarologia* **11:** 484–503.

Statgraphics (1998). *Statgraphics Plus for Windows*, version 4.0 user's guide. *Statistical Graphics*, Rockville, MD.

Tagawa, J. (1987). Post-mating changes in the oviposition tactics of the parasitic wasp, *Apantales glomeratus* L. (Hymenoptera: Braconidae). *Appl. Entomol. Zool.* **224:** 537–542.

Tillman, P. G. (1994). Age-dependent parasitization and production of female progeny for *Microplitis croceipes* (Hymenoptera: Braconidae). *Southwest. Entomol.* **19:** 335–338.

van den Assem, J. (1974). Male courtship patterns and female receptivity signal of Pteromalinae (Hym., Pteromalidae), with a consideration of some evolutionary trends and a comment on the taxomic position of *Pachycrepoideus vindemiae. Neth. J. Zool.* **24:** 253–278.

van den Assem, J. (1986). Mating behavior in parasitic wasps. In Waage, J., and Greathead, D. (eds.), *Insect Parasitoids*, Academic Press, London. pp. 137–167.

van den Assem, J. (1996). Mating behavior. In Jervis, M., and Kidd, N. (eds.), *Insect Natural Enemies—Practical Approaches to Their Study and Evaluation*, Chapman & Hall, London, pp. 163–221.

van den Assem, J., and Povel, G. D. E. (1973). Courtship behaviour of some *Muscidifurax* species (Hym., Pteromalidae): A possible example of a recently evolved ethological isolating mechanism. *Neth. J. Zool.* **23:** 465–487.

van den Assem, J., Jachmann, F., and Simbolotti, J. H. (1980). Courtship behaviour of *Nasonia vitripennis* (Hym., Pteromalidae): Some qualitative, experimental evidence for the role of pheromones. *Behaviour* **75:** 301–307.

van den Assem, J., and Werren, J. H. (1994). A comparison of the courtship and mating behavior of three species of *Nasonia* (Hym., Pteromalidae). *J. Insect Behav.* **7:** 53–66.

Vinson, S. B. (1972). Courtship behavior and evidence for a sex pheromone in the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). *Environ. Entomol.* **1:** 409–415.

Willers, R. L. J. (1999). Three fatalities involving phosphine gas, produced as a result of methamphetamine manufacturing. *J. Forens. Sci.* **44:** 647–652.

Zar, J. H. (1984). *Biostatistical Analysis*, Prentice Hall, Englewood Cliffs, NJ.