

Emergence, Mating, and Postmating Behaviors of the Oriental Beetle (Coleoptera: Scarabaeidae)

Henry T. Facundo,^{1,2} Charles E. Linn, Jr.,¹ Michael G. Villani, and Wendell L. Roelofs^{1,3}

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*In a previous field-trapping study of the oriental beetle, *Exomala orientalis* (Waterhouse), by using synthetic sex pheromone on golf course fairways, numerous males were observed and trapped during the hours of peak mating activity. However, very few beetles were observed in the same areas when synthetic pheromone was absent. To investigate the hypothesis that mating in nature occurs cryptically within vegetation at the soil surface, laboratory studies on female emergence and pheromone release, male emergence and mate-locating, and female and male mating behaviors were conducted. Mate acquisition and copulation occurred on the soil surface near the female emergence site, with both sexes engaging in pheromone-mediated behaviors after having emerged from the soil. A highly stereotyped female pheromone release, or calling, behavior was observed, consisting of insertion of the female's head into the soil and elevation of the tip of her abdomen into the air. Bioassays conducted in a wind tunnel that simulated a turf fairway environment showed that walking and flying were both important in the upwind response of males to females. Mating and copulation occurred without an obvious complex courtship, but observations of postmating behaviors suggested that mate guarding occurs.*

KEY WORDS: Coleoptera; Scarabaeidae; oriental beetle; *Exomala orientalis*; sustained-flight tunnel; sex pheromone; (*Z*)- and (*E*)-7-tetradecen-2-one; mating behavior; mate guarding.

¹ Department of Entomology, Barton Laboratory, New York State Agricultural Experiment Station, Cornell University, Geneva, New York 14456.

² Current address: Department of Entomology, University of the Philippines, Los Baños, College, Laguna 4031, Philippines.

³ To whom correspondence should be addressed.

INTRODUCTION

The mating ecology of the oriental beetle, *Exomala orientalis* (Waterhouse), an important turf and woody plant pest in the northeastern United States, is characterized by a relatively short mating season [2 months in the latitude of New York, NY (Facundo *et al.*, 1999; Hallock, 1930)], with an adult life span of about 2 weeks (Bianchi, 1935; Facundo, unpublished laboratory data). Mate location occurs via a sex pheromone, produced and released by the female (Leal, 1993; Zhang *et al.*, 1994). In an initial field investigation on three golf course fairways (Facundo *et al.*, 1994), thousands of male oriental beetles were caught daily in traps baited with the synthetic pheromone components (Z)- and (E)-7-tetradecen-2-one. However, when synthetic pheromone was not present, adult activity was seldom observed in the same fairways, even during the peak hours of activity around sunset (Facundo *et al.*, 1994; Facundo, 1997). Females were rarely observed irrespective of the presence of synthetic pheromone.

These field observations suggested that sex pheromone-mediated mate-locating behaviors of the oriental beetle occur at or below the soil surface and may not involve long-range flight. We tested this hypothesis by analyzing emergence and mate-locating behaviors of males and females in simulated turf environments in the laboratory. The mating sequence and postmating associations between the mated pair and competing males also are described.

MATERIALS AND METHODS

Insects. Second- and third-instar *E. orientalis* were collected from the Green and Black golf courses at Bethpage State Park, Farmingdale, NY (40°45'N, 73°28'W), in September 1995 and in April and September 1996. Individual grubs were placed in 30-ml plastic cups with ca. 25 g of moist ($\approx 14\%$, w/w, unless otherwise noted) loamy sand soil and approximately 1 g of grass seeds and held at 10°C in constant dark to slow development. When adult beetles were required, batches of grubs were transferred to a 25°C chamber with a photoperiod of 16:8 (L:D) h, as needed, and checked weekly for pupae. Late-stage pupae were inspected daily, without disturbance, for adult eclosion (day 0) to document the age of each adult.

Emergence Behaviors. Newly eclosed adults (days 0 to 2) were placed individually in 300-ml emergence cups (10-oz Solo Ultra Clear; Solo Cup Company, Urbana, IL; 10 cm in height, 7.5 cm in mouth diameter), which were filled with 250 g of sieved loamy sand soil and topped with ≈ 1 cm of transplanted Kentucky blue grass, *Poa pretensis*, sod. This sod was sprinkled with white sand (Lime Crest Tropical Play Sand, Limestone Products Corp., Sparta, NJ), to contrast with the tan and black adults. Cups were then videotaped top-view, one or two at a time, using a General Electric closed circuit television 4TE44BSA117, a

Panasonic time-date generator WJ-810, a Panasonic VHS video cassette recorder AG-1240, and a Sony Trinitron KV-1393R monitor. Videotaping was done in a walk-in environmental chamber with settings intended to approximate summer field conditions (Facundo *et al.*, 1999): 20°C from 0200 to 0600 h, rising to 25°C by 1400 h, staying at 25°C until 1800 h, and decreasing to 20°C by 0200 h; lights on from 0600 to 2200 h; and a relative humidity of 40–60%. During the scotophase, a lamp with a 25-W red bulb above the arena provided sufficient light to videotape beetle activity. An airflow of 40 ± 10 cm/s at the emergence cup was achieved with an electric fan (Model BFC2000; Essick Air Products, Little Rock, AR) 2.5 m from the arena. A 2.5-cm feather hung near the mouth of the cup detected changes in wind direction. A vaneometer (Dwyer Instruments, Inc., Michigan City, IN) monitored wind speed.

Videotaping was continuous until the beetle emerged and left the cup or had not emerged after 14 days. Videotapes were first viewed to define distinct behaviors that occurred upon emergence from the soil. The tapes were then reviewed to determine the frequency and duration of each behavioral state. The objective was to determine whether males and females exhibited pheromone-related behaviors at, and immediately after emergence, in the presence or absence of two relevant cues, wind and pheromone. The treatments and the number of males tested were as follows: no wind + no pheromone (6 males), wind + no pheromone (13 males), and wind + pheromone [septum with 10 μ g, 95:5 (Z:E); 5 males]. For females, there were two treatments with five females each: no wind and wind. The following nonparametric tests were used in the analyses (Conover, 1980): Cox and Stuart test to detect trends in the occurrence of each behavior, Kruskal-Wallis test (for males) and Mann-Whitney test (for females) to compare the behavior parameters measured among treatments, and chi-square test to check nonrandomness in directions taken by calling females. For this experiment and those described below, reported values are mean \pm standard error (SE); unless otherwise noted, $\alpha = 0.05$.

Mate-Locating Behaviors. Five trays of sod (1.69 m long, 0.45 m wide, 7 cm above the tunnel floor) were arranged inside the wind tunnel (Miller and Roelofs, 1978) to simulate a turf environment. Tests were conducted within the first 4 h of scotophase (10–15 lux, 20–22°C, 40–60% RH, and tunnel wind speed of 35 ± 5 cm/s). Adult beetles were acclimated to the wind tunnel room conditions for ≈ 1 h before a test. The source, three virgin females (4–8 days old) inside a screen cage (2 \times 5 \times 5 cm), was placed on a wire mesh platform (10 \times 10 cm) positioned on top of the sod at the upwind end of the turf. Virgin males ($n = 34$; 4–8 days old) were introduced sequentially at the downwind end of the turf on a second wire-mesh platform, 1 m downwind of the females. Care was taken not to agitate the insects during introduction. Each male was allowed 3 min to respond to the pheromone.

The response of males was analyzed to (1) determine the success rate of

males in locating the female source, (2) to identify distinct behaviors associated with mate location, and (3) to quantify the variability in the pattern of these behaviors, to identify key steps that resulted in success or failure. Sequence analysis was performed following Krasnoff and Roelofs (1990), with the degree of variability for each behavioral transition determined by calculating the “stereotypy index” developed by Haynes and Birch (1984). This index provides an objective measure of the variation in the probabilities of the behaviors observed following each behavior. A stereotypy index for the overall sequence of behaviors was also calculated and is the average of the values for all transitions weighted according to the number of males observed to exhibit the behavior. For recurring acts (those that occur more than once throughout the sequence, such as cleaning antennae), 10 s of alternative activity was arbitrarily selected as the minimum interval separating two distinct bouts. The durations of each behavioral state were compared among conditions using the Kruskal–Wallis test.

Mating and Postmating Behaviors. For the analysis of behavior during the final stages of mate location, copulation, and postcopulation, we used a dissecting microscope (Wild M3Z; Heerbrugg, Switzerland) equipped with a NEC Color CCD Camera NX18AS and Panasonic time–date generator WJ-810, connected to a Sharp TV/VHS-VCR combination 25VT-G100. An electric fan was positioned 2.5 m from the setup to provide a steady stream of air (40 ± 10 cm/s at the sod). All experiments were conducted between the last 2 h of the photophase and the first 4 h of the scotophase of the rearing cycle at $\approx 21^\circ\text{C}$ with lights on and augmented with microscope lights as needed for video recording. The time and temperature combination was chosen because they are representative of the peak period of activity in the field (Facundo *et al.*, 1994, 1999).

The mating arena was a $7.5 \times 5.0 \times 1.5$ -cm piece of sod, chosen to simulate a turf environment. A virgin female (≥ 4 days after eclosion) was placed on top of the mating arena and allowed to acclimate and initiate release of pheromone (also referred to hereafter as calling; see Results, below). Treatments consisted of either one ($n = 25$) or two ($n = 21$) males (≥ 4 days posteclosion), placed inside a small screen cage(s) and positioned >5.0 cm from the downwind side of the sod. Males were allowed to become active and walk upwind to track the plume of the pheromone released by the female. Prior to an experiment, each beetle's weight was determined using a Mettler balance (AE100; Hightstown, NJ), so as to provide a comparative ranking of sizes. Beetle weight is positively correlated with length ($r = 0.61$ and 0.85 for males and females, respectively; $n = 30$ for both) and width ($r = 0.61$ and 0.71 , respectively; $n = 30$ for both).

All activities were video recorded and later analyzed as described above for emergence and mate-locating behaviors. A chi-square test for independence was used to analyze the possible factors or behaviors associated with success or failure of the second male to mate with the mated female (Conover, 1980; StatSoft, 1985).

RESULTS

Emergence. Both male and female beetles exhibited pheromone-related behaviors immediately upon emergence from the soil. These behaviors did not involve flight but, rather, were confined to the soil surface and were dependent on the presence of wind and pheromone (males) or wind (females).

Males. Behaviors identified upon emergence from the soil were head out, antennae splayed to the air (Fig. 1A); body out, male continuing to splay his antennae; activates, vigorous waving of the antennae and forelegs upon detection of the pheromone (Fig. 1B); leaves arena, walking off the arena (7.5 cm diameter); cleans antennae, folding the antennae and passing the flagellum (lamellae) through the mouthparts and sometimes through the forelegs' tibial and tarsal spines (Fig. 1C); faces new direction, moving to a direction $\geq 20.0^\circ$ from the previous direction the male was facing, whether or not the movement was within or around the emergence hole or to a new spot; and walks to a new spot, movement from an arbitrarily defined area of 2-cm radius from the emergence hole (or, for later movement, from the new spot).

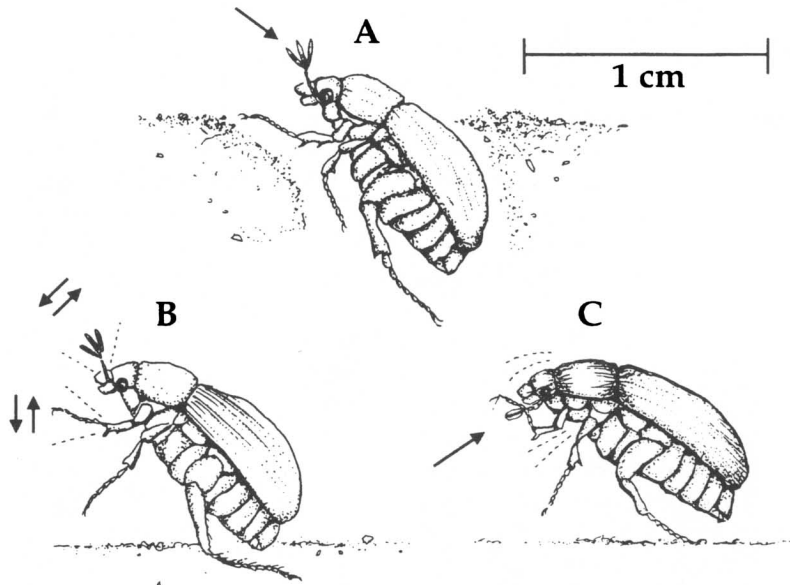


Fig. 1. Illustration of behaviors associated with male emergence from the soil. (A) The male's head is out of the soil, his antennae splayed and directed upwind; (B) the male becomes active upon detection of pheromone (the forelegs are raised and waved vigorously together with the antennae); and (C) the male cleans his antennae by passing the folded lamellae through the mouthparts and the spines on the foretibiae and tarsi.

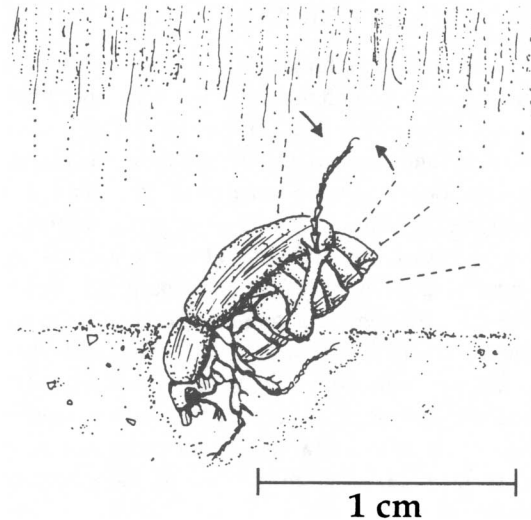


Fig. 2. A female in the calling position, with the head inside the emergence hole and only the abdomen exposed to the air. Leg movements illustrate leg raising and brushing over the apical segments of the abdomen, where the pheromone glands are located. See text for details.

In the presence of wind and pheromone 100% of the males displayed the sequence from head out to leaves arena, the entire sequence lasting 1.3 ± 1.0 min, significantly shorter than residence times in the arena of 257.6 ± 117.6 min with wind-pheromone and 118.3 ± 49.1 min with no wind or pheromone (Kruskal-Wallis test). Recurrent behaviors were common in the no-wind and no-pheromone test, with males cleaning their antennae every 11.6 ± 1.5 min, changing direction every 12.7 ± 2.3 min, and moving to a new spot every 91.2 ± 36.9 min.

Females. The identified behaviors of females upon emergence from the soil were head out, antennae splayed; body out, antennae also splayed; calling position (Fig. 2), inserting her head back into the emergence hole and exposing only the abdomen, elevating it by extending the hindlegs when they are not used in leg raising and brushing; raises at least one hindleg and then makes a downward stroke(s) over the tip of the abdomen (Fig. 2); faces new direction, changing the abdomen's orientation ($\geq 20.0^\circ$); cleans antennae; walks to new spot; and leaves arena, as described for males.

When a female broke through the sod and left the emergence hole, she exhibited a highly stereotypic head stand posture at that site (Fig. 2). We refer to this posture as "calling," in keeping with many other pheromone studies, and presume that the posture is related to release of the sex pheromone. Although not tested directly by collection of airborne material, evidence in support of this con-

clusion comes from mating studies (below) in which males approached and initiated mating with females 100% of the time when females were in this posture. Occasionally, rhythmic anteroposteriad “pumping” or pulsation of the abdomen was visible. Another recurrent act was hindleg raising and stroking; the female raised either (or both) hindleg above the caudal end of the elytra and/or the exposed tergites and then stroked it (them) down with varying speed. In general, during these strokes, the leg did not touch any part of the abdomen. At the raised position, occasional dorsoventrad movements of the pretarsus (waving up–down) were observed.

Female calling behaviors were significantly affected by the presence of wind, with females remaining in the emergence spot for a significantly longer time in the presence than in the absence of wind (500.5 ± 121.4 versus 183.5 ± 74.2 min; Mann–Whitney test). In the absence of wind females either left the emergence spot for a new spot in the arena or left the arena altogether. Females changed direction (in which the abdomen tip was facing) every 7.0 ± 1.1 min in the wind, compared with 15.4 ± 1.8 min in the absence of wind (Mann–Whitney test). No linear trends were detected for the occurrence of antennal cleaning, leg raising and stroking, direction changes, and transfers to new spots throughout the duration of each individual’s stay in the observation arena (Cox and Stuart test for trends).

Mate Location. The male behaviors identified in the wind tunnel (Fig. 3) were splays antennae, spreading the lamellae; activates, vigorous waving of antennae and forelegs; walks upwind, upwind ($<90^\circ$ in either direction relative to the direction of the tunnel wind) movement ≥ 1 cm; walks downwind, walking ≥ 1 cm downwind ($>90^\circ$ in either direction relative to the direction of the wind); climbs grass blade, staying longer than 2 s; attempts flight, spreading the hindwings and attempting to fly; flies upwind, upwind flight ($<90^\circ$ in either direction relative to the direction of the wind) for ≥ 1 s; goes back to soil or leaves arena; and finds source.

In wind tunnel conditions simulating a turf environment, with live females as a pheromone source, 100% of the tested males ($n = 34$) became active and initiated upwind-directed movement by walking instead of flying. As in the emergence studies above, males became active by splaying their antennae in the air, spreading the lamellae and vigorously waving the antennae and forelegs. Fifty-three percent of the males reached the source, with 61% of these males exhibiting a combination of walking and flying ($\chi^2 = 6.28$, $P = 0.01$). In no case did a male make a complete flight to the female source. As indicated by the complexity of transitions (Fig. 3), the low stereotypy indices for some of the transitions (0.23 for walks downwind, 0.34 for climbs grass blade, 0.50 for flies upwind), and the low overall stereotypy index of 0.68, there was considerable variability in the sequence of behaviors exhibited by males, following the initial steps, activation and walking upwind. Bouts of walking occurred in both the upwind and

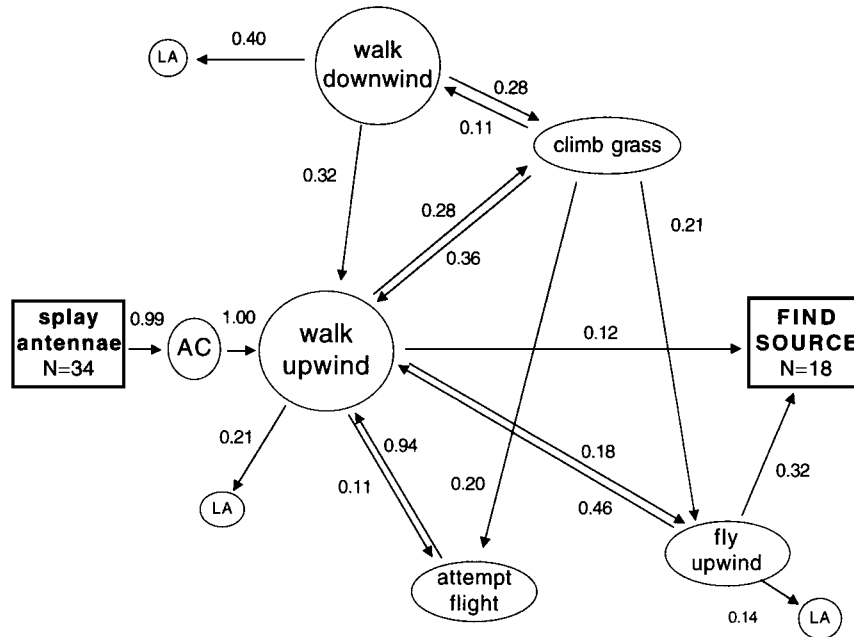


Fig. 3. Kinematic graph showing conditional probabilities of first-order transitions between behavioral states associated with mate location by males using three virgin females as a source ($n = 34$). Numbers above arrows represent the conditional probabilities of each transition. Probabilities may not sum to 1.0 because of rounding or because frequencies < 0.10 are not shown. Boxes are initial and final states of the sequence with the number of individuals at each state. Behavioral states are as indicated; AC = activates and LA = leaves arena. A stereotypy index of 0.68 was calculated for the overall sequence. The stereotypy index = $\sqrt{\{(\sum P_{ij})^2 - (\sum P_{ij})^2/r_i\}/(1 - 1/r_i)}$, where P_{ij} is the probability of transitions from behavior i to all "following" behaviors (j). The number of possible transitions from the "preceding" behavior (i) is indicated by r_i .

the downwind directions, with males sometimes climbing tall grass blades and remaining there longer than 2 s with their antennae splayed in the air and facing in an upwind direction, prior to taking flight, attempting to take flight, or continuing to walk in an upwind or downwind direction. Males left the arena following several behaviors, and thus there was no key step involved in a male's success or failure in locating the female source (Fig. 3). Males that only walked took significantly longer to reach the source than those that walked and flew in some combination (684.0 ± 212.3 for walking only versus 149.4 ± 26.8 for walking and flying; one-tailed Mann-Whitney test, $P = 0.0016$).

Mating: Female and Single Male. When the female was in the head-stand posture, the mating sequence involving a single male (Fig. 4) was highly stereotypic as evidenced by the conditional probability values in Fig. 5, the range of high stereotypy indices for the behavioral transitions in Fig. 5 (0.87 to 1.00),

and the overall stereotypy index value of 0.92. There was no evidence of a courtship exchange between male and female; rather males typically approached the female at the caudal end, touching the pygidium first (Fig. 4A), then immediately mounted by climbing on top of the female (Fig. 4B). Once on the female, the male grasped the lateral edges of her elytra with his forepretarsi, the proximal end of the hindtibiae with his mesopretarsi, and the hindtarsi with his hindpretarsi. The male then moved his body posteriorly while arching his abdomen forward to insert his aedeagus into the genital chamber of the female (Fig. 4C). With the aedeagus inserted and the female with the anterior half of her body in the soil, the frequency of movements decreased and the pair stayed in this position for an average of 21.8 ± 2.0 min. After this, the first movement observed, in general, was that of the male when he retracted his aedeagus from the female's genital chamber by raising his abdomen away from the female while still holding her with his fore- and midlegs (Fig. 4D). The female immediately started to move down into the soil and the male released her (Fig. 4E). After releasing the female the male left the spot (Fig. 4F).

Mating: Female and Two Males. When two males approached a female, the first male to reach the female copulated with her in all 21 observations. When the second male approached, the first male, using his hindlegs, held the second male while in copula. The first male released the second male only after releasing the female, and after the female was completely buried in the soil. If the second male displaced the first, the mating sequence was initiated again and could involve holding off the first male as the latter tried to retake his lost position. The presence of a second male did not significantly affect the time from insertion to retraction of the aedeagus (21.8 ± 2.0 min for one male versus 21.9 ± 2.6 min for two males), but there was a significant difference in the time from retraction of the aedeagus to release of the female (0.7 ± 0.1 min for one male versus 20.1 ± 5.8 min for two males).

In the 21 tests involving two males, 39 attempts were made to dislodge the first male (12 males made more than 1 attempt), with 6 successful takeovers. These takeovers occurred because the first male did not hold the second male continuously ($n = 2$) or did not guard the female by continually holding her while she was still aboveground ($n = 4$). There was no significant relationship between the difference in weights of the competing males and a successful takeover ($\chi^2 = 0.09$, $P = 0.77$).

The three most common maneuvers employed by the second male to dislodge the first male (Fig. 6) were (1) to climb on top of the first male and insert his aedeagus (Fig. 6A; $n = 13$); (2) to go between the pair through the anterior, mid-section, or posterior part of the pair (Fig. 6B; $n = 16$); and, (3) to position himself in front of the first male, holding onto the anterior half of the female using the forelegs and midlegs while pushing the first male posteriorly using his body and hindlegs (Fig. 6C; $n = 10$). In all three maneuvers, the first male generally

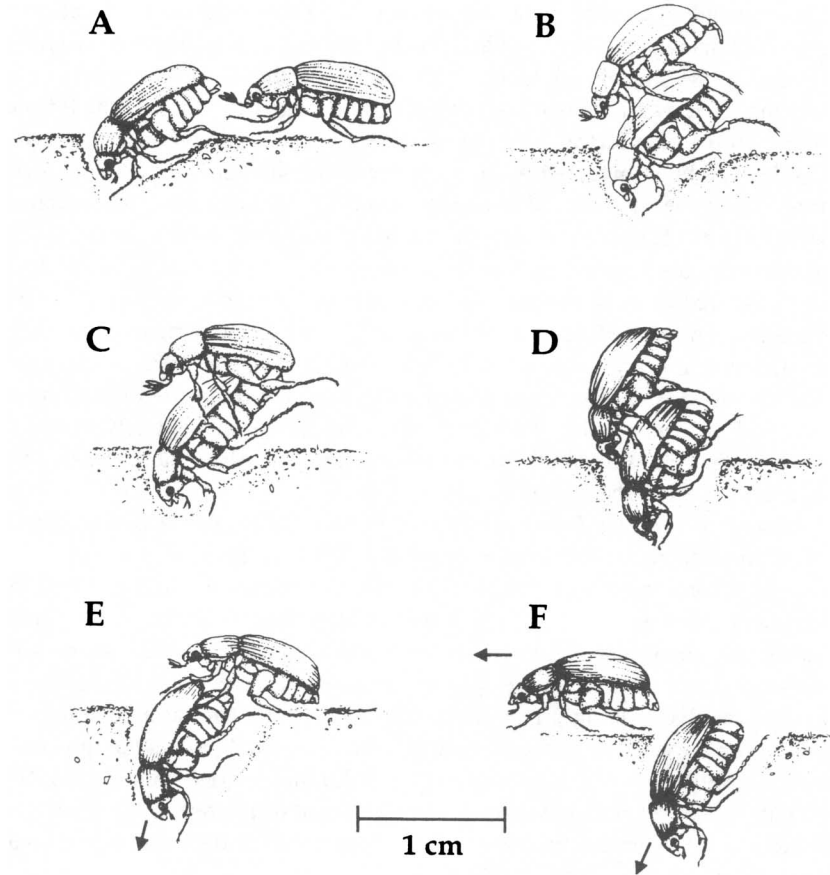


Fig. 4. Illustration of behaviors in the mating sequence of the oriental beetle. (A) The male approaches a female; (B) the male mounts the female and starts to position his legs for securing the female's body; (C) the male inserts his aedeagus into the female's genital chamber; (D) the male retracts his aedeagus from the genital chamber by raising his abdomen up and holding the female with his two front pairs of legs; (E) the female moves down to the soil while the male dismounts but still holds her posterior parts; and (F) the male leaves the spot while the female continues to dig deeper.

continued to copulate and grasp the female and, at the same time, hold one of the legs of the second male, attempting to push the latter's body off the female.

DISCUSSION

Our results support the hypothesis that mate-locating behaviors in the oriental beetle occur at or close to the soil surface and that this accounts for the

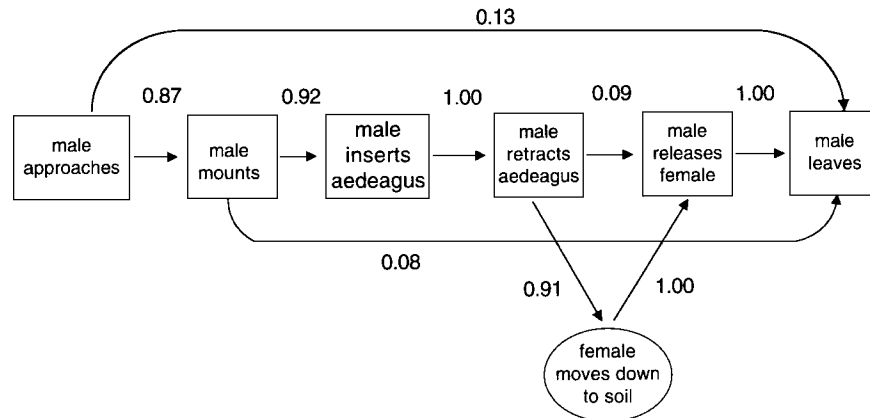


Fig. 5. Kinematic graph of the mating sequence of the oriental beetle showing conditional probabilities (values next to arrows) of first-order transition between behavioral states, from Fig. 4. Only those observations when the female had inserted her head in the thatch when the male reached her are included ($n = 25$). A stereotypy index of 0.92 was calculated for the overall sequence (see Fig. 3).

smaller than expected number of insects seen flying during the hours of peak activity (Facundo *et al.*, 1994; Tashiro, 1987; Bianchi, 1935). First, in the presence of wind and/or pheromone, males and females exhibited pheromone-mediated behavior on the soil surface immediately upon emergence. Second, females called (released pheromone) after assuming a stereotypic head-stand posture at the site of emergence from the soil. Third, males responded to the pheromone in the wind tunnel by a combination of walking and flying short distances over the surface of the turf. Our results also show that mating did not involve a complex courtship and that there was evidence for postcopulatory mate-guarding.

Pheromone Release by Females. The head-stand posture observed for female *E. orientalis* has been observed in other beetles, including males of the scarabs *Kheper aegyptiorum* (Sato and Imamori, 1986, 1988) and *K. nigroaeneus* (Edwards and Aschenborn, 1988), males of the burying beetle *Nicrophorus vespilloides* (Eggert, 1992), and females of the dermestids *Trogoderma variable* (Cross *et al.*, 1977) and *T. glabrum* (Hammack *et al.*, 1976). In all of these beetles, pheromone glands have been demonstrated, or hypothesized based on behavioral observations, to be associated with the abdominal sternites. The head-stand behavior presumably enhances airborne dissemination of the pheromone downwind from the position of the beetle on the surface of the ground.

Female *E. orientalis* also exhibited leg raising and stroking, which may aid in the dissemination of the pheromone by generating air turbulence at the release sites or function as an auxiliary dispenser of the pheromone. Males of *K.*

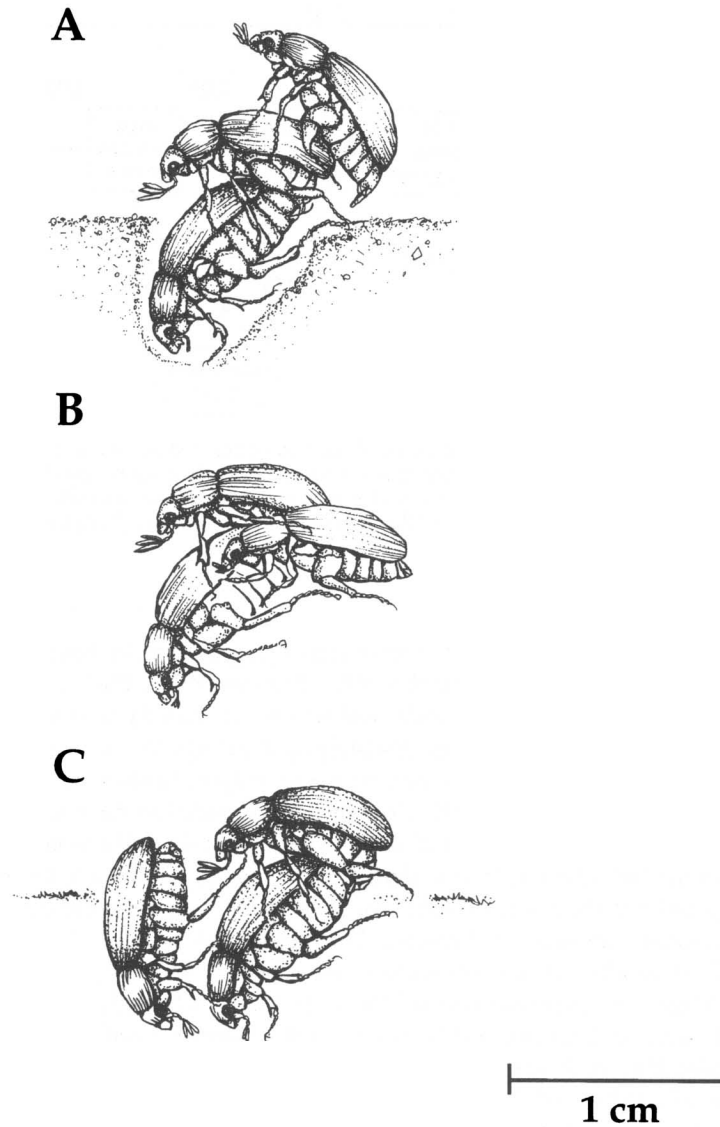


Fig. 6. Illustration of three maneuvers of a second male while the first male was guarding the mated female. (A) The second male mounts the copulating first male and tries to insert his aedeagus into the genital chamber of the female. (B) The second male tries to dislodge the copulating first male by inserting his head (and then his body when successful) between the pair. (C) The second male mounts the anterior half of the female and tries to dislodge the copulating first male by pushing the latter back using his hindlegs.

nigroaeneus and *K. aegyptiorum* also display leg movements while assuming the head-standing posture. Male *K. nigroaeneus* rub the hindleg tibial brushes along the ventral surface of the abdomen to release a pungent white powder containing the pheromone (Edwards and Aschenborn, 1988). In *K. aegyptiorum*, Sato and Imamori (1986) characterized "hindleg bending behavior" as alternately withdrawing and extending the hindlegs while the male stood on his head. The leg movements of these species differ from that of a calling oriental beetle female, whose hindlegs only occasionally touched the abdomen and were swept dorsally over the pygidium and down to the space between the substrate and the apical sternites, rather than just along the ventral surface of the abdomen.

Male Response to Pheromone. Zhang *et al.* (1994) reported significant numbers of beetles exhibiting upwind flights to synthetic pheromone over a 1.5-m distance in the wind tunnel. These tests utilized the same protocol that has been successfully used with many moth species (Linn and Roelofs, 1989), involving upwind flight over a distance from 1 to 1.5 m in an airborne filamentous plume generated by a semiturbulent airflow. The results of Zhang *et al.* (1994) demonstrated that male *E. orientalis* can exhibit sustained optomotor anemotactic flight, similar to many moth species and at least one other scarab beetle (Domek *et al.*, 1990). In the present study our objective was to test the hypothesis that mate location takes place on or near the soil surface, and thus wind tunnel conditions were modified to simulate a turf environment at dusk, with female sources placed on the surface of the turf. Dusk is the time of peak mating activity (Facundo *et al.*, 1994), and the preferred calling position of females is on the soil surface. Our wind tunnel results show that these modifications had a significant effect on male behavior, with males exhibiting a more complex behavior pattern that included bouts of walking and flying. Walking occurred in both the upwind and the downwind directions and often was associated with periods when males stopped, climbed a blade of grass, and splayed their antennal lamellae to the air, possibly sampling the airspace. While the overall sequence displayed considerable variability, with very few instances of sustained directed movement to the female source, the behaviors are similar to those seen in nature at dusk in situations without synthetic pheromone (Facundo *et al.*, 1994, 1999).

Male behavior in our simulated turf environment may have been significantly influenced by the structure of the airborne pheromone signal close to or at the turf surface. In this situation the spread of the pheromone downwind may be more complex than in the relatively structured filamentous plumes that can occur in the open air (Murlis, 1997), with short plumes of pheromone-laden filaments interspersed over the turf with more diffuse areas of pheromone and/or pheromone-free air. One hypothesis is that flying occurred in the presence of a filamentous plume structure, with walking occurring in more diffuse areas of pheromone or when the pheromone signal was lost. Our results show that only

53% of the males successfully reached the source, indicating that males frequently lost the pheromone signal. This further stresses the point that in nature mate location may take place in a very restricted area around the emergence site.

Male behavior in the wind tunnel also may have been affected by environmental conditions such as temperature and/or light intensity. Males were tested at 10–15 lux and 20–22°C, conditions that are characteristic of twilight conditions. This contrasts with the wind tunnel study by Zhang *et al.* (1994), in which beetles were tested at 27–28°C and 800 lux. Observations made by Facundo *et al.* (1999) on the smaller numbers of beetles present in golf course fairways during the warm parts of the day showed that beetles exhibited longer and higher altitude flights, although not necessarily in response to pheromone. This was in contrast to the time around sunset, when the surface temperature was cooler and when more males were observed, either stationary or walking. Whether cooler temperatures were energetically unfavorable for sustained flight by males is questionable, however, as many males were trapped and observed flying during the dusk activity period when synthetic pheromone was present on gold fairways (Facundo *et al.*, 1994). The combined observations indicate that the behavior of adult males can display considerable plasticity, depending on movement of the pheromone over the turf surface and/or environmental conditions.

Comparison of the wind tunnel response of male *E. orientalis* with that of other beetle species is difficult, as this subject has not been investigated in detail for any species. Wind tunnel studies have been conducted with the European elm-bark beetle, *Scolytus multistriatus* (Choudhury and Kennedy, 1980), and the furniture beetle, *Anobium punctatum* (Birch and White, 1988), as well as the green June beetle, *Cotinis nitida* (Domek *et al.*, 1990). Many scarab species have been captured in pheromone-baited traps (Leal, 1988; Facundo *et al.*, 1994, 1999; Zhang *et al.*, 1997; unpublished results from our laboratory with several other species of *Phylophaga*), in both nocturnal and diurnal settings, again demonstrating that males can exhibit odor-modulated anemotaxis over long distances. Whether this is part of the natural mating behavior, however, may depend on other factors, such as with *E. orientalis*, where females restrict their calling behaviors to the soil surface.

Mating and Postcopulatory Mate-Guarding Behaviors. There was no evidence for an elaborate courtship between male and female *E. orientalis*, rather successful mating appears dependent largely on a male simply finding a female that is in the calling position. Two behaviors were observed that could possibly be associated with courtship. First, when the female was deep in the thatch, the male vigorously touched her dorsum by using his legs and mouthparts. Second, while in copula, males were sometimes observed to stroke the anterior part of the female's elytra by using the maxillary and labial palpi, accompanied by head bobbing. Males of the scarabs *Onthophagus binodis* and *Phanaeus daphnis* use

their forelegs to tap or drum the dorsum of females, accompanied by pulsation of the male's abdomen. Besides the similar tapping of the female's dorsum (mostly by the mouthparts), video analyses revealed that abdominal pumping and quivering of the male's body occur in *E. orientalis* while in copula. These behaviors may be associated with the label copulatory courtship (Eberhard, 1991), a form of cryptic female choice (see Thornhill, 1983), but we caution that we have no direct evidence that the tapping behavior alters copulatory success or affects the fitness of female *E. orientalis* in any way.

There was evidence of postcopulatory mate guarding in *E. orientalis*. In the presence of a second male the inseminating male maintained physical contact with the female for a significantly longer period and aggressively rejected the second male's attempts to copulate. This is the first reported case of mate guarding in a scarab beetle that does not involve parental care, such as *Kheper platynotus* (Sato and Hiramatsu, 1993) and *Onthophagus binodis* (Cook, 1988). Mate guarding also occurs in the burying beetle *Nicrophorus orbicollis* (Scott, 1990), a species whose mating system similarly involves parental care (Eggert *et al.*, 1998), the staphylinid beetles *Ontholestes cingulatus* (Alcock, 1991), *L. eistotrophus versicolor* (Alcock and Forsyth, 1988), and *Eumicrota* spp. (Ashe, 1987), several chrysomelids (Dickinson, 1997), the curculionid *Cosmoploites sordidus* (Viana and Vilela, 1996), the cerambycid *Anoplophora chinensis* (Wang *et al.*, 1996), several tiger beetles (Shivashankar and Pearson (1994), the scolytid *Ips pini* (Reid and Roitberg, 1994), and the meloid *Nemognatha nitidula* (Brown and Stanford, 1992).

Demonstrated, or suggested, functions of mate guarding in the various beetle species noted above are that it increases paternity confidence and, in cases where parental care is involved, provides assistance and protection to the female and her offspring. Although these do not apply to *E. orientalis*, several predictions for the mate-guarding hypothesis proposed by Alcock (1994) appear to be satisfied. These include (1) a male-biased operational sex ratio (Facundo *et al.*, 1994, 1999), (2) a brief period between copulation and oviposition (Tashiro, 1987), during which the females stay in the soil, and (3) mated females remaining receptive to additional mates (Facundo, 1997). Other predictions that may be applicable to this insect, but have not been tested, are that sperm precedence is high, risk of injury and predation while guarding is low, energy expenditure while repelling rivals is low, access to mated females is high, and the refractory period (to replenish ejaculate) is long.

The oriental beetle's mating system can be aptly placed in the category of scramble competition polygyny (Thornhill and Alcock, 1983). In this scenario advantage goes to the male that can get to the female first and keep other males from replacing him during copulation and before the female starts to oviposit. Mate guarding in *E. orientalis*, thus, probably functions entirely to prevent other males from copulating with a female. With a relatively short adult life of approx-

imately 2 weeks, multiple matings by males may be the exception rather than the rule. Protandry, also observed in *E. orientalis* (Facundo *et al.*, 1999), may be an additional evolutionary response of males to the intense competition for a female.

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