

# Attraction and learning in mate-finding by solitary bees, *Lasioglossum (Dialictus) figueresi* Wcislo and *Nomia triangulifera* Vachal (Hymenoptera: Halictidae)

William T. Wcislo\*

Snow Entomological Museum, Department of Entomology, University of Kansas, Lawrence, KS 66045-2119, USA

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**Summary.** Males of the solitary sweat bee, *Nomia triangulifera*, patrol over large areas where thousands of females emerge, searching for receptive females. The daily operational sex ratio is strongly male-biased. Males contact dead, frozen (untreated) females more frequently than they contact females which were washed in hexane, showing that olfactory cues are utilized in mate-finding. A major source of female sex pheromone is in the head. Male pouncing on females is temporally non-random, indicative of group stimulation. Bioassays show that newly emerged females are more attractive to males than are older pollen-collecting females. Female odors are individually distinctive, based on male responses, and there is much variation among females in their attractive properties. Male responses to female odors suggest that learning is important for mating in natural populations. In contrast, the following hypotheses are unlikely to account for the observed behavior: (1) dissipation of female odors; (2) site learning and avoidance behavior by males; (3) decay of male motivation; or (4) male-produced repellents effective against other males. Laboratory and field studies show that female *Lasioglossum figueresi* produce individually-distinctive odors, which are attractive to males. There is considerable inter-individual variation among females in their attractiveness to males among sexually immature females. Male responses to female odors decay over the course of the presentation, suggesting the importance of learning in natural populations, although several alternatives could not be tested.

## Introduction

Female bees, wasps, and ants (Hymenoptera, Aculeata) are well-known for their sophisticated learning abilities (see von Frisch 1967; Hölldobler and Lindauer 1985;

Fletcher and Michener 1987). In contrast, the influence of prior experience on male behavior is little known, despite suggestions from Darwin (1871) and others that learning might be important in sexual selection (Robson and Richards 1936; Lloyd 1980; West-Eberhard 1983). Barrows (1975a, b) and subsequent authors showed that males of an eusocial bee, *Lasioglossum (Dialictus) zephyrum* (Smith) (Halictidae), habituate to odors of nest-mate females, and of unreceptive females, and modify their behavior in ways appropriate to the situation, under both laboratory and natural conditions (Greenberg 1982; Smith 1983; Smith and Ayasse 1987; Wcislo 1987; reviews: Michener and Smith 1987; Greenberg 1988).

These studies seemingly support the hypotheses of various authors that male learning by hymenopteran species represents an evolutionary origin for the recognition systems which permit cohesive kin-based social groups (Hölldobler and Michener 1980; Michener 1982; Michener and Smith 1987; Greenberg 1988; also Jacobs 1924, cited in Linsley 1958). In particular, since males can learn odors of female nest-mates, they later avoid the same or similar odors to prevent inbreeding, and also avoid very dissimilar female odors to prevent extreme outbreeding [after Bateson 1983; for genetic consequences, see Lynch (1991); Serradilla and Alaya (1983)]. Male learning of individual odors also might reduce mating effort expended on previously-encountered unreceptive females (Wcislo 1987; Michener and Smith 1987).

Almost nothing is known, however, about learning by males in solitary species or populations. This paper reports experiments on a solitary bee, *Nomia triangulifera* (Nominae), which certainly represents a pre-social level of evolution, and the bee *Lasioglossum (Dialictus) figueresi* (Halictinae), which might be secondarily solitary, based on morphological comparisons with its relative, *L. (D.) aeneiventris* (Wcislo 1990). The objectives are to (i) ascertain whether or not males respond to and learn individually different female-produced odors under semi-natural conditions; (ii) determine the sources

\* Present address: Department of Entomology, Cornell University, Ithaca, NY 14853, USA

of female-produced odors; and (iii) study the role of social facilitation among males.

## Materials and methods

### Natural history

*Nomia triangulifera*. Mating behavior was studied at large nesting aggregations on a farm along the Kansas River (Douglas Co., Kansas). The number of nesting females varied from about 50000 to over 150000 (between 1987 and 1990), and roughly equal numbers of males and females were produced (details in R.L. Minckley, W.T. Wcislo, D. Yanega, and S.L. Buchmann, in preparation). *N. triangulifera* is active from mid-August through September in northeastern Kansas, and experiments reported here were conducted between 1984 and 1987.

Populations were protandrous. After emerging, males flew close to the ground in great numbers over areas where females emerged, and were also abundant on plants (*Helianthus annuus* L.) from which females gather resources. Males pounced on females, and displayed elaborate pre-copulatory behavior (Wcislo et al., in press a). They sometimes briefly pounced on other males or small wasps. Approximately 2–3 weeks after emergence, males became scarce at the site and on sunflowers. Voucher specimens are in the Snow Entomological Museum, University of Kansas (SEMUK).

*Lasioglossum figueresi*. These bees nested in vertical earthen banks along trails near San Antonio de Escazú, Costa Rica (details in Wcislo et al., submitted b). Female and male brood were present in equal numbers. Adults emerged to mate in mid- to late-June during a characteristic lull of the wet season. Males patrolled among bushes along a vertical earthen bank opposite the one where females nested (the banks were about 3 m apart, and the length of the patrolled area was about 20 m). Males became progressively less abundant throughout July, and were rare by the end of the month (1988).

Flight patterns were less frenetic than those of smaller *L. (Dialictus)* (Batra 1966; Michener and Wille 1961; pers. obs.). A male approached a female (Fig. 1, left), flying a serpentine path. Males pounced on females that landed on a leaf, twig, or the bank, and the pair then fell to the ground ( $n=17$ ). In one case, mating was brief (43 secs), as in some other *L. (Dialictus)* (Barrows 1975b), with no courtship other than copulatory vibrations. Males also sometimes pounced on other males, wasps, etc. Voucher specimens are in SEMUK, the Instituto Nacional de Biodiversidad (Heredia, Costa Rica), and elsewhere (Wcislo 1990).

### Emergence patterns and patrolling behavior in *Nomia triangulifera*

The potential for intra-sexual competition was estimated by measuring the daily operational sex ratio [OSR: the number of receptive females emerging on a date, divided by the cumulative number of males emerging to date, assuming males live 13 days (which represents the mean longevity of females)]. Emergence phenologies (1989 and 1990) were determined by placing three  $1 \times 0.5$  m and one  $1 \times 1$  m emergence traps (W.T. Wcislo and R.L. Minckley, in preparation) over areas where bees nested the previous year. Traps were checked daily, the numbers and sexes of emerging bees were recorded, and the bees were removed.

There was yearly variation in the locations and sizes of the areas where females emerged, and where males patrolled, but this reflected agricultural land-use patterns. Results from a preliminary mark-recapture study show that some males remained within the area (either  $9 \text{ m}^2$  or  $100 \text{ m}^2$ ) where they were first marked (see Wcislo 1991). No marked males were recaptured at distances greater than 100 m, despite the fact that most probably flew much further to visit sunflowers for nectar. Consequently, I assumed



Fig. 1. Left: a male *Lasioglossum figueresi* (body length about 6 mm) approaches a pinned, unextracted female (cropped from photograph); middle: a male attempts necrophilic copulation with an unextracted female; right: several males climbing on another male attempting to mate with an unextracted female

that there were different sub-populations of males at different ends of the fields.

### Role of male learning of female odors in *Nomia triangulifera*

Putative female sex pheromones were studied by comparing the attractiveness to males of frozen untreated females versus hexane-washed females. Emerging females were collected in an insect net, and transferred to individual 6-dram glass vials. Vials were carried to the laboratory on ice, and placed in a freezer to kill the bees; they are called *unextracted females* below ( $n=20$ ). An additional 20 female bees, collected and killed as described above, were given 3 washes in excess hexane, baked in a  $50^\circ \text{C}$  oven for 24 h, and then placed in individual vials. These *extracted females* were presumably devoid of any volatile chemicals. The extraction protocol did not obviously change the visual appearance of females. Vials were stored overnight in a freezer, and then carried to the field the next day on ice in a cooler. Females were coded so that during tests and video analyses I did not know their class.

A test involved taking a frozen female from a vial, sticking a new #2 or #3 insect pin through her thorax, and then inserting the pin in the soil so that the bee was 2–3 cm above the surface. Responses of males were videotaped using a tripod-mounted Panasonic® camera. Videos were scored by counting the numbers of males approaching and contacting the pinned female for each minute during a 5-min period.

A *contact* was unequivocal, and varied from a brief touch, to extended copulation attempts. *Approaches* were obvious when the

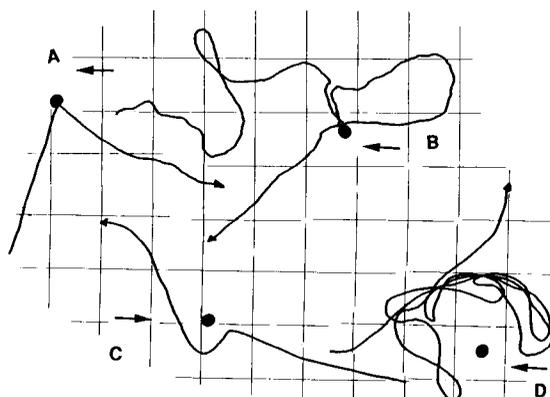


Fig. 2. Digitized flight paths from video recordings (August 1986) of male *Nomia triangulifera* responding to frozen unextracted females (black circles). Arrows indicate wind direction. Flight paths A and B involve approaches and contacts, while paths C and D involve only approaches

male faced the female and flew near (<5 cm) her, hovering or flying along a serpentine to semi-circular flight path (after Kullenberg 1973; Smith 1983; Wcislo 1987). Representative flight paths of approach and contact (Fig. 2) were made by copying a video image to a computer with a digitizing bit-pad (input at 11 data points/sec). Approach becomes more subjective in distinguishing some cases (e.g., Fig. 2C) from a male just flying by; more emphasis is placed on biological interpretations of rates of contact. Furthermore, most males were not marked, so it was not possible to distinguish between one male contacting a female twice, and two different males doing so once each [see Hirsch and Holliday (1988) for a criticisms of using group data to study learning].

Male learning of individual female odors was studied by presenting unextracted females ( $n=30$ ) to males at two sites, A1 and B1 (order of presentation determined by a coin flip), each for 5 min; these bees were presented a second time at A and B (indicated by A2 and B2), and then at a third site C (note the order of testing was not blind; methods from Wcislo 1987). Sites A and B were approximately 100 m apart on a line roughly parallel to the direction of the prevailing winds, and site C was >500 m away at the other end of the farm. Male behavior was scored for each minute of a 5-min period. An additional 10 unextracted females were left exposed to air (away from males) for 30–60 min before they were presented to males to determine if putative attractants dissipated over time at field temperatures (29–31°C).

To determine if male response wanes with time, another 34 frozen unextracted females were presented to males at the same site, and data were recorded and analyzed as above.

#### *Localization of Nomia triangulifera female odors and age-related variability*

To localize the source of potential pheromones, the head, mesosoma (thorax), and metasoma (abdomen) of an unextracted female ( $n=30$ ) were separated using clean razor blades, and each tagma was individually crushed immediately before testing onto clean  $2 \times 4$  mm black velvet rectangles. These rectangles were then tested as for unextracted females ( $n=30$  for each group of tagmata).

Results indicated that unextracted females differed in their attractiveness to males. To determine if differences were related to age, I compared the attractiveness of newly emerged females, with older females already provisioning nests. Emerging females ( $n=15$ ) were collected and prepared as above, and pollen-carrying females were captured as they returned to their nests ( $n=15$ ; each female was tested at three sites, and there were no significant differences among sites, so results were pooled to give  $n=45$ ). Pollen-carrying females were conspicuously yellow, so these tests were not blind. To determine if the yellow color of pollen influenced male response, an additional five pollen-collecting females were likewise obtained and frozen. As much pollen as possible was removed with an insect pin and a fine brush, and the bees were thoroughly washed in several changes of distilled water, and returned to the freezer.

#### *Social facilitation among males of Nomia triangulifera*

To determine if the activity of other males influences patrolling males, randomly chosen 1-min periods of video from 25 unextracted females were divided into 60 events each (25 periods; total number of 1-s events=1500). An event was scored as "yes" if a male approached the female during that time, and as "no" if a male did not approach the female. Males that remained and courted the dead female were counted only once.

#### *Cage experiments on individual odors in Lasioglossum figueresi*

Male and female pupae from different natal nests (collected in mid-April) were transferred to plastic tissue culture trays, with several pupae per plastic cell. All bees, therefore, had social experi-

ence following eclosion. Young (callow) adults were kept together for 48 h and periodically fed honey-water (1:1). A total of 19 unextracted female bees and 11 extracted bees were prepared as described for *N. triangulifera*. Also 11 males (48–96 h post-eclosion) were transferred to an inverted mesh "fish cage" ( $16 \times 12 \times 12$  cm), and were given honey-water *ad libitum* (methods in Greenberg 1982).

To determine if males respond to females' odors, their behavior was compared when they were presented with unextracted or extracted females. Coded females were pinned, and individually placed in the cage with males (food removed), which was placed outdoors in bright sunlight in still air. Approaches and contacts were counted using hand-held counters for each minute during 5 min.

#### *Field studies on mate attraction and learning in Lasioglossum figueresi*

Some experimental studies required several groups of naive males. I located one population of active males, and mark-recapture studies (Wcislo 1991) showed that males did not restrict their flight paths to a subset of the area where males occur, unlike some other *Lasioglossum* (*Dialictus*) species (Kukuk 1989 and reference therein). Consequently, it was not appropriate to assume that there were different sub-populations of males, precluding certain experiments.

Experiments on learning used protocols described for *Nomia*, with modifications. Adult female bees ( $n=20$ ) were collected from nest excavations at one aggregation, placed in individual glass vials and killed by freezing. Frozen bees were stored overnight in a freezer, and the next day were carried in a cooler with "ice substitute" (Coleman's Chillers) to another nest aggregation ("Near site" and "Nice view" of Wcislo et al., in press b). Females were pinned and placed at the ends of branches of bushes along the bank where males patrolled (Fig. 1, middle); this site was mid-way between the ends of the patrolled area. Responses were scored per minute for 4-min periods, and again not all males were marked. An additional 5 unextracted females were left exposed to air (away from males) for 30–60 min before presentations to males to determine if putative attractants dissipated after exposure to air (23–27°C).

#### *Statistical considerations*

Different *N. triangulifera* test sites were chosen to have approximately the same densities of patrolling males, but these were not quantified. In order to correct for differences in male densities, the number of contacts per female was divided by the approaches to that female. This measure of "attractiveness" (contacts/approaches =  $C/A$ ) is a ratio, and approaches and contacts are not independent, so an arcsine transformation seems biologically inappropriate. The data resulting from these experiments should be viewed in this light (from Wcislo 1987).

Statistical tests are indicated in the text, and were taken from Conover (1971) or Sokal and Rohlf (1981), or from Statview on a Macintosh computer. Unless indicated otherwise, means are given with their standard errors.

## Results

### *Operational sex ratio (OSR) in Nomia triangulifera*

Males began emerging in mid- to late-August, and emergence continued for about 2 weeks. Several days later females began emerging, and thereafter male and female emergence periods overlapped. The population sex ratio varied from equality to slightly male-biased (R.L.

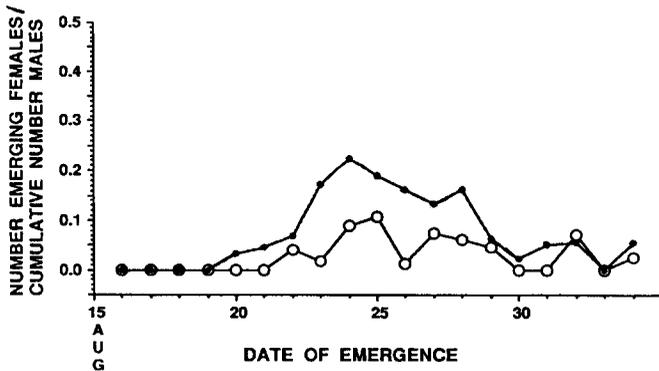


Fig. 3. Operational sex ratio (OSR) per day during the mating season for *Nomia triangulifera* for 1989 (black dots) and 1990 (white circles)

Minckley et al., in preparation), while the daily OSR was always heavily male-biased, even at peak female emergence (Fig. 3). Consequently, males probably compete for receptive females.

#### *Nomia triangulifera* female odors as sexual releasers

Male response to female visual stimuli was increased by the presence of female odors, as shown by comparing the mean number of contacts per approach ( $C/A$ ) on frozen unextracted females [ $\bar{x}=0.7 \pm 0.15$  (SD),  $n=30$ , for site A1] versus extracted ones [ $\bar{x}=0.1 \pm 0.1$  (SD),  $n=20$ ] ( $P < 0.001$ , Mann-Whitney  $U$  test). Young females, not yet carrying pollen, were contacted more frequently per minute ( $\bar{x}=5.6 \pm 0.76$ ,  $n=45$ , pooled for first presentations of 15 females at each of 3 sites) than older females already carrying pollen ( $\bar{x}=1.1 \pm 0.18$ ,  $n=45$ ) ( $P < 0.0001$ ,  $t=4.6$ ,  $t$ -test; also significant with Mann-Whitney  $U$  test). These young females were also contacted more frequently than pollen-collecting females with their pollen removed [ $\bar{x}=1.2 \pm 0.24$ ,  $n=15$  (5 females at 3 sites)] ( $P < 0.0001$  with  $t$ -test or Mann-Whitney  $U$  test). These findings suggest that the color of pollen does not deter males (the odors associated with lipids from the pollenkitt could mask female odors, but were not controlled). A comparison of male responses to black squares with crushed heads of young and old females gives similar results (below). The differential male responses, therefore, are probably not due to the yellow pollen, but to female maturational changes. In numerous hours of observations for other studies, pollen-laden females ( $>100$ ) always rejected courting males.

Bioassays show that a major source of the sex pheromone is in the females' heads. The mean number of contacts for "Nomia heads" of newly emerged females was  $8.0 \pm 0.69$  ( $n=30$ ), greater than the number of contacts to the corresponding "Nomia abdomen" ( $\bar{x}=3.5 \pm 0.43$ ,  $n=30$ ) ( $P < 0.0001$ ,  $t=7.9$ , paired  $t$ -test). Both of these values were greater ( $P < 0.0001$ ; paired or unpaired  $t$ -tests) than responses to either "Nomia thoraces" ( $\bar{x}=1.3 \pm 0.22$ ,  $n=30$ ) or a blank black velvet square ( $\bar{x}=1.0 \pm 0.19$ ,  $n=15$ ). Heads from females that already were

collecting pollen were contacted less frequently ( $\bar{x}=2.1 \pm 0.53$ ,  $n=10$ ) than those from females not yet collecting pollen (above;  $P < 0.05$ , Mann-Whitney  $U$  test).

#### The role of learning by male *Nomia triangulifera*

Based on unextracted newly emerged females, the mean  $C/A$  for the first presentation at site A is slightly more than those at B1 and C ( $P=0.011$ ), and there were no significant differences between B1 and C ( $P > 0.4$ , Wilcoxon Sign Rank test) (Fig. 4). Differences in the numbers of approaches between these sites were not significant ( $P > 0.2$ ). The mean  $C/A$  for A1 was significantly greater than for the second presentation at the same site A2 ( $P=0.004$ ). In contrast, at site B there was a slight increase in mean  $C/A$  between first (B1) and second (B2) presentations, but the difference was not significant ( $P > 0.5$ ). These mixed findings indicate that al-

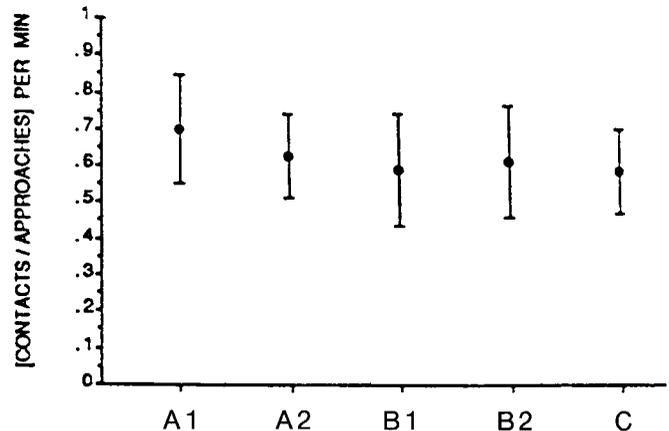


Fig. 4. Mean ( $\pm$ SD) number of contacts per approach for the same unextracted females presented twice at sites A and B, followed by presentations at site C

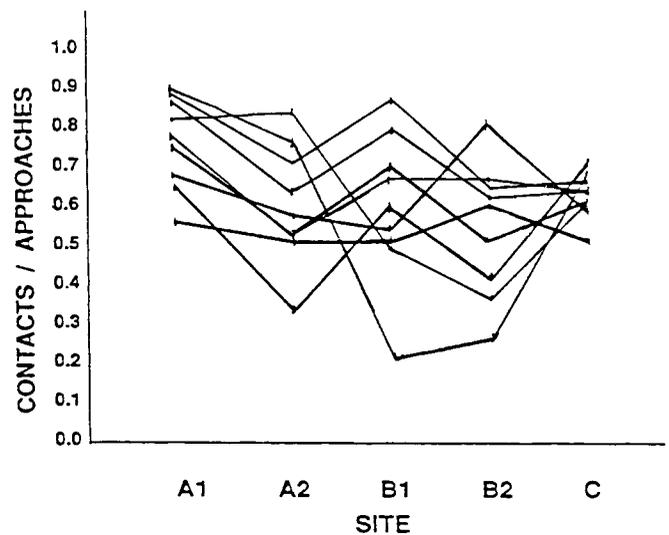


Fig. 5. Line graphs showing the contacts/approach by males towards individual frozen unextracted female *Nomia triangulifera* for repeated presentations of the same females at different sites

though male learning may occur, its biological significance may be swamped by other factors (see Discussion). Examples showing individual variability in female attractiveness at different sites and presentations is given in Fig. 5.

The videotaped responses of males to frozen females ( $n=34$ ) over time at a single site (B) are summarized in Fig. 6. These analyses show that the number of males approaching unextracted females was not significantly different throughout a 5-min period (Fig. 6, top). The number of contacts (Fig. 6, middle) and the number of contacts/approaches (Fig. 6, bottom), however, were significantly less for the second minute compared to the first following initial presentation (both  $P=0.002$ ), and still less for the third minute ( $P=0.001$ ), but thereafter were not significantly different ( $P>0.05$ ).

The decay in responsiveness of males to unextracted females can be interpreted with one of the following hypotheses (modified from Wcislo 1987): (1) female odors dissipate rapidly and are less pronounced during

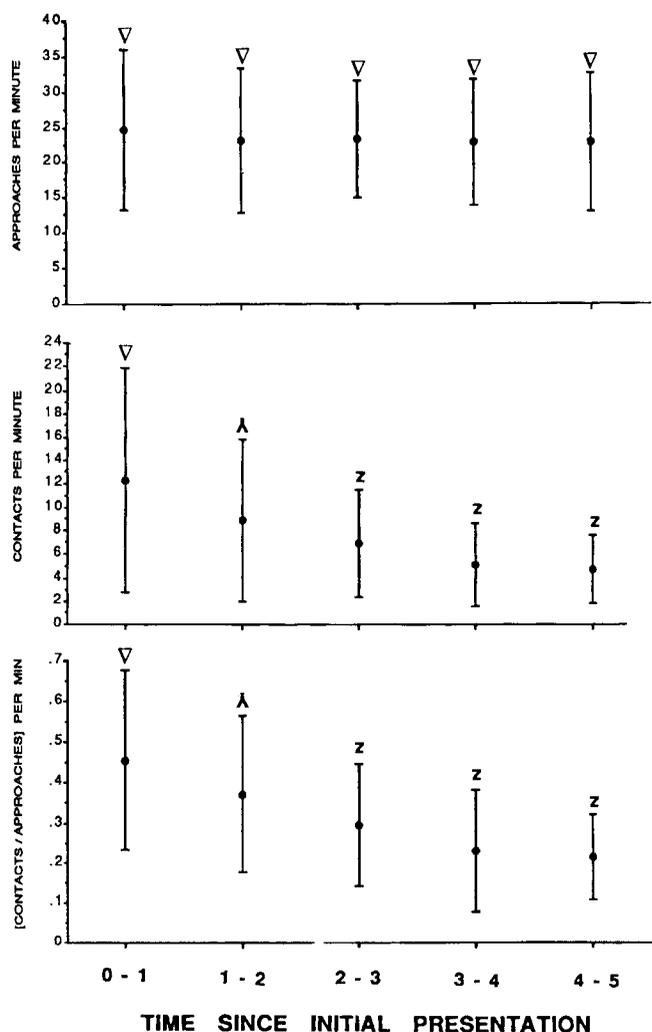


Fig. 6. The mean ( $\pm$  SD) number of approaches (top), contacts (middle), and contacts/approach (bottom) by male *Nomia triangulifera* presented with unextracted females. Within each graph, values not sharing characters are significantly different at  $P<0.05$ . Data are based on analyses of videotape. Time is min

subsequent presentations; (2) males learn to avoid the places at which unreceptive females are presented; (3) males learn that unextracted females are not sexually receptive and during subsequent encounters they tend not to respond to those learned odors; (4) male motivation decays spontaneously after initial activation (if females are perceived as a class); (5) males deposit individually distinctive chemical badges enabling them to recognize and ignore already contacted females (assuming males do not respond to the badges of other males); or (6) males mark females with repellents ("antiaphrodisiacs") that deter other males prior to intromission (cf. Kukuk 1985).

Females presented at site C remain attractive to males, despite repeated contacts from other males, so hypotheses related to dissipation of odors (1) and male repellents (6) can be eliminated. Furthermore, unextracted females left exposed to the air ( $<60$  min) were contacted [mean  $C/A=0.67\pm0.21$  (SD)] at rates similar to females kept on ice ( $P>0.3$ ). Site learning (2) and motivational decay (4) can be eliminated since males pounced on novel females presented at the same site. Most males were not individually marked, so the significance of male recognition badges (5) cannot be determined on the basis of this experiment.

#### Social facilitation among male *Nomia triangulifera*

Habituation can be broken by novelty, or by apparent novelty, if individuals pay attention to the behavior of others that are responding directly to a stimulus. The importance of this simple group effect was determined by detailed analyses of video tapes. Records of 25 one-minute periods are summarized in Fig. 7. Tested individually, in 6 of these 25 periods the distributions of approaches deviated from random ( $P<0.05$ ), and overall the pooled data are significantly non-random (Wald-Wolfowitz runs test,  $T=510$ ,  $P<0.05$ ). The temporal distribution of pounces suggests that males respond to the activity of other males, in addition to their responses to female odors. Males infrequently contact frozen or otherwise dead (untreated) males (unpublished data), nor do they usually pounce on other flying males, even

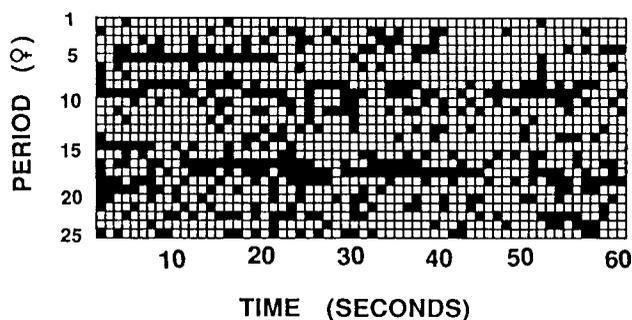


Fig. 7. Results for 25 one-minute periods in which each second was treated as an event with two possible outcomes: a male approaches (black box) or a male does not approach (white box) a pinned, unextracted female *Nomia triangulifera*. ■ ♂ approach to unextracted ♀; □ no ♂ approach to unextracted ♀

**Table 1.** Responses of caged male *Lasioglossum figueresi* to unextracted or extracted frozen conspecific females

	Female contacted?	
	Yes	No
Unextracted ♀	17	2
Extracted ♀	1	10

$P < 0.0001$ , Fisher's exact test, and Pearson's Phi

when the latter are near the source of female odor, although occasional homosexual courtships occurred.

#### Olfactory sexual releasers in *Lasioglossum figueresi* (cage experiments)

In small laboratory flight cages newly eclosed adult males pounced on unextracted females more frequently than they did on extracted ones (Table 1). The presence of olfactory cues reduced a male's threshold to respond to other cues, even though newly eclosed males and females in nature are not sexually active.

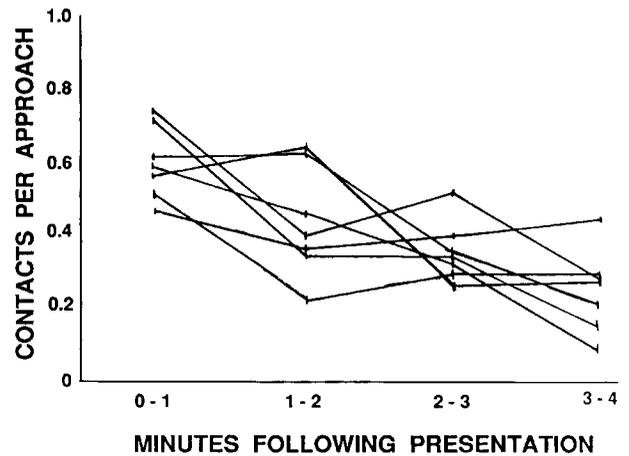
The rate of male pouncing on dead females (0–5 contacts per 5-min period) was much lower than that observed under natural conditions, and may relate to sexual maturation of the males or females. Ovaries of these females were slender. Two frozen unextracted females were not contacted by males. Of the 17 contacted females, 8 were sufficiently attractive to cause the male to evert his genitalia and remain mounted on the dead female for 12–244 s [ $\bar{x} = 73 \pm 73.9$  s (SD),  $n = 10$  males]. Males did not perform any visible courtship behavior.

#### Field studies on olfactory sexual releasers and learning in *Lasioglossum figueresi*

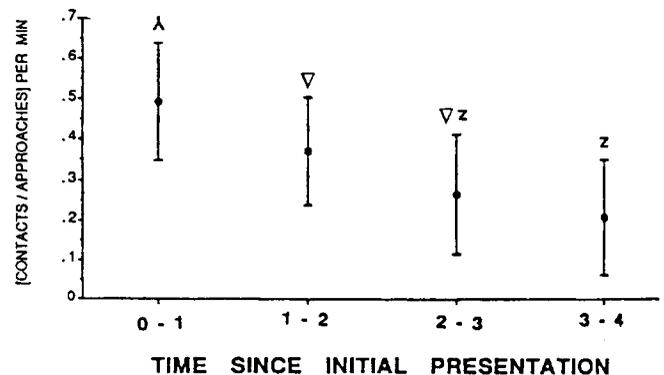
Males left their natal nests in mid-June, and (at least at one site) patrolled among vegetation along an earthen bank where females nested. They began flying at 0900 hours on clear, sunny days, and continued to fly until mid-afternoon when it became cloudy or rained. Males were not obviously aggressive to one another while flying, but vigorously pushed other males which were crawling on frozen females (Fig. 1, right).

Free-flying males were attracted to females when stimulated by chemical cues, and readily attempted to copulate with models (Fig. 1, middle). The mean number of contacts/approaches per minute for all presentations ( $n = 76$ ) of unextracted females was greater [ $\bar{x} = 0.35 \pm 0.17$  (SD)] than the rate in cage experiments. In this population there is much variability in that some females were frequently contacted and some infrequently contacted (Fig. 8).

The mean ( $\pm$ SD) number of contacts per approach (C/A) for *L. figueresi* is given in Fig. 9, for each of 4 min during a presentation. The mean C/A for the first minute



**Fig. 8.** Line graphs showing the contacts/approaches by males towards individual unextracted female *Lasioglossum figueresi*



**Fig. 9.** The mean ( $\pm$  SD) number of contacts/approach for male *Lasioglossum figueresi* presented with the odors of unextracted females. Values not sharing the same characters are significantly different at  $P < 0.017$ . Time is min

( $\bar{x} = 0.49$ ) was significantly greater than the C/A for the second minute ( $T_s = 14$ ,  $P = 0.001$ , Wilcoxon sign rank test); there is a trend towards decreasing response in the remaining minutes, but the decreases were not significant.

The decay in responsiveness of male *L. figueresi* to unextracted females may stem from any of the hypotheses given for *N. triangulifera*. For *L. figueresi* several of these possibilities could not be tested, since only one population of males was available, and individuals patrolled over the entire site. Males sometimes remained on females for long periods of time, and males displayed such behavior throughout trials, so it is unlikely that females *per se* became progressively less attractive (hypothesis 1). Females exposed to air for  $< 60$  min were as attractive to males [C/A,  $\bar{x} = 0.46 \pm 0.12$  (SD),  $n = 15$ ] as were females that were frozen until the time of testing ( $P > 0.5$ , Mann Whitney  $U$  test). Dissipation of odors, therefore, is probably not responsible for the decay in responsiveness. Site learning (hypothesis 2) and motivational changes (hypothesis 4) are probably not important since the presentation of novel unextracted females again

attracted males. It is, therefore, possible to eliminate hypotheses 1, 2 and 4, while those related to male-produced badges (5) and repellents (6) could not be tested, and cannot be rejected.

## Discussion

The following conclusions emerge from this study: (1) male solitary bees, *Nomia triangulifera* and *Lasioglossum (Dialictus) figueresi*, are sexually attracted to female odors; (2) bioassays using male behavior show that females produce individually distinctive odors; (3) for *N. triangulifera* a major source of the odors is a cephalic gland, and the attractive properties change with age or mated status of the females (cf. Engels and Engels 1988); (4) male learning of individual female odors is the better supported hypothesis to account for changes in male behavior toward female odors in *N. triangulifera*, and is one of three possible hypotheses remaining for *L. figueresi*; (5) females vary in their attractiveness to males, or males vary in their responsiveness to females; and (6) males of *N. triangulifera* increase their sexual pouncing behavior in the presence of other active males, indicative of social facilitation.

### Mate attraction

Results from studies of both *L. figueresi* and *N. triangulifera* show that males more frequently respond to female visual stimuli in the presence of olfactory cues. In general, male bees and sphecoid wasps will pounce on small, dark objects (dots, dead bees, and so forth) that are female-sized, especially in the presence of female odors (e.g., Barrows 1975a; Evans and O'Neill 1986, 1988; Kullenberg 1973; Tengö and Bergström 1977; Tengö 1979; Bergmark et al. 1984; Free 1987; Tengö et al. 1988; Shimron and Hefetz 1985; for vespoid wasps, Keeping et al. 1986; Ono and Sasaki 1987). Inferential evidence suggests there is scrambling competition among males for receptive females (see Alcock et al. 1978). At least for solitary species and populations, it seems unlikely that such conditions would generate selection for refined discriminating abilities by males (i.e., rejecting receptive females).

The relative importance of visual and olfactory cues for mate recognition is known for only a few apoids (see Eickwort and Ginsberg 1980). For *N. triangulifera*, the number of males contacting an odorless (extracted) female was not significantly greater than the number contacting a black rectangle, suggesting that visual cues alone do not provide specific stimulation. Similarly, black models with crushed female heads (appropriate olfactory cue + inappropriate visual cue) were contacted more frequently than were extracted whole females (no olfactory cue + appropriate visual cue). Models with the large scopae of female *Dasygaster altercator* were more attractive to conspecific males than were models without the scopae, and models with odor were

more attractive than models without odors (Bergmark et al. 1984; also see Butler 1965; Barrows 1975b; Meyer-Holzappel 1984; Koeniger 1990). In most cases, a variety of cues seem to provide information which enables males to recognize and respond to sexually receptive females as rapidly as possible.

### The significance of male learning for mating behavior

Responses of male *L. figueresi* and *N. triangulifera* to unextracted females at a single site resemble a waning response expected under habituation: soon after the initial presentation, the responsiveness of males gradually decays. Habituation to individual odors of unreceptive females occurs in a social bee (Barrows 1975a; Greenberg 1982; Wcislo 1987; Smith and Ayasse 1987), and effectively serves to decrease mating effort (*sensu* Low 1978) invested in previously-encountered unreceptive worker females [see Robacker et al. (1976) for an example in parasitic wasps]. This effect of habituation is significant for bees since under natural conditions numerous females are not receptive, and males probably compete for access to mates (e.g., Batra 1966). Habituation by males (Thorpe 1963; Peeke and Herz 1973; Peeke and Petrinovich 1984), in combination with female nesting philopatry, should result in intermediate levels of outbreeding (see Wcislo 1987).

### Social facilitation and apparent female variability

The responses of different males to individual female odors are temporally non-random, suggesting that males respond to movement of other males. Increased response of reacting individuals is indicative of social facilitation ("group stimulation") (e.g., Allee 1938; Clayton 1978). Stochastic fluctuations in the density or distribution of patrolling males, coupled with learning, may be amplified in unpredictable ways (cf. Allen and Sanglier 1978; Deneubourg et al. 1987), and locally exacerbate intra-sexual competition [see Wade and Pruett-Jones (1990) for consequences of "female copying" behavior]. Such behavior might also help explain some of the inter-individual variability in apparent female-attractiveness (also Wcislo 1987). Additional sources of variation include differences due to imprecision or noise in the neural control of behavior (Jander 1968; Eberhard 1990), individual variability in sensory systems (Christensen et al. 1990; see Miller et al. 1960), or the weather (Larsson 1989).

Variability in female attractiveness implies that some females might be less likely to mate than others because they are less likely to attract partners (see Gadagkar et al. 1990 for vespoid wasps). Among recently emerged *L. figueresi*, males in a flight cage were attracted to some females but not others. These differences might be especially relevant for social bees since a discriminating male mating with a future gyne will have higher average fitness than one mating with a worker. Female variability

in attractiveness (“desirability”) to males, however, has been described for a fly, *Drosophila silvestris* (Spiess and Carson 1981), and may be widespread among animals (see Williams 1959).

### *Mating, learned recognition, and social evolution*

Learning abilities by males of solitary aculeates have been hypothesized as one source of variation for the origin of nest-mate recognizing abilities in social species (e.g., Hölldobler and Michener 1980). In *N. triangulifera*, *L. figueresi*, and many other species, males compete with other males (Alcock et al. 1978; Thornhill and Alcock 1983). Under these conditions selection for refined discriminating abilities is expected to be stronger in females than in males [possible exceptions include *Dianthidium ulkei* (Frohlich and Parker 1985), and *Halictus ligatus* (Packer 1986)]. Although there may be some cost to mating repeatedly (e.g., decreased longevity), as known for abnormal and normal *Drosophila* females (Bellen and Kiger 1987; Fowler and Partridge 1989), the typically biased operational sex ratio (OSR) suggests that few males naturally have opportunities to mate themselves to death. Competition among males should create selection for abilities to rapidly habituate to cues associated with unreceptive females, coupled with selection against discriminating among the smaller class of receptive females. The latter should diminish as the direct fitness of receptive females becomes more variable, as for social species (Packer 1986). In such situations, females producing discriminating sons might realize higher average fitness. In general, however, learning enables males to decrease time spent with females known to be unreceptive from previous experience. Zawistowski and Richmond (1985; also Zawistowski 1988) reach a similar conclusion from studies of male learning in *Drosophila*. As with the bees, the majority of female fruitflies in nature at a given time are not receptive (e.g., Gromko et al. 1980).

Female bees put much effort into rearing offspring, and males do not. This asymmetrical investment implies that females will be the discriminating sex (see Trivers 1972; West-Eberhard 1983; Wade 1987), but little is known about female mate-choice in bees (see Wcislo et al., in press b). In some Apoidea (e.g., *Xylocopa*) males release volatile chemicals to attract females, which fly from one territory to another, and eventually mate with a male (e.g., Minckley et al. 1991). If females make comparisons, then they must learn some features of a male's phenotype for comparisons with subsequent male(s), creating conditions under which sexual selection can operate for more refined female discriminatory abilities (for a general discussion, see Wcislo 1989). If sexual selection is considered, especially in light of the natural selection on females for nest recognition abilities (Hölldobler and Michener 1980; Wcislo, in press), then an elaboration of the female sensory system represents a more plausible origin for nest-mate recognition by female Hymenoptera than a sex-related origin which assumes that males learn in order to discriminate among

receptive females, accepting some and rejecting others (see Ratnieks 1991).

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### References

- Alcock J, Barrows EM, Gordh G, Hubbard LJ, Kirkendall LL, Pyle D, Ponder TL, Zalom FG (1978) The ecology and evolution of male reproductive behaviour in the bees and wasps. *Zool J Linn Soc* 64:293–326
- Allee WC (1938) The social life of animals. Abelard-Schumen Ltd, NY
- Allen PM, Sanglier M (1978) Dynamic model of urban growth. *J Soc Biol Struct* 1:265–280
- Barrows EM (1975a) Individually distinctive odors in an invertebrate. *Behav Biol* 15:57–64
- Barrows EM (1975b) Mating behavior in halictine bees (Hymenoptera: Halictidae): III. Copulatory behavior and olfactory communication. *Insectes Soc* 22:307–322
- Bateson P (1983) Optimal outbreeding. In: Bateson P (ed) *Mate choice*. Cambridge University Press, Cambridge, pp 367–377
- Batra SWT (1966) The life cycle and behavior of the primitively social bee, *Lasioglossum zephyrum* (Halictidae). *Univ Kansas Sci Bull* 64:359–423
- Bellen HJ, Kiger JA Jr (1987) Sexual hyperactivity and reduced longevity of *dunce* females of *Drosophila melanogaster*. *Genetics* 115:153–160
- Bergmark L, Borg-Karlson A-K, Tengö J (1984) Female characteristics and odour cues in mate recognition in *Dasypoda altercator* (Hym., Melittidae). *Nov Act Reg Soc Sci Upsaliensis (Ser V) C*:137–143
- Butler CG (1965) Sex attraction in *Andrena flavipes* Panzer (Hymenoptera: Apoidea), with some observations on nest-site restriction. *Proc R Entomol Soc London (A)* 40:77–80
- Christensen TA, Geoffrion SC, Hildebrand JG (1990) Physiology of interspecific chemical communication in *Heliothis* moths. *Physiol Entomol* 15:275–283
- Clayton DA (1978) Socially facilitated behavior. *Q Rev Biol* 58:373–392
- Conover WJ (1971) *Practical nonparametric statistics*. John Wiley & Sons, New York
- Darwin C (1871) *Sexual selection and the descent of man* (1981 reprint). Princeton Univ Press, Princeton
- Deneubourg J-L, Goss S, Pasteels JM, Fresneau D, Lachaud J-P (1987) Self-organization mechanisms in ant societies (II): learning in foraging and division of labor. *Exper Suppl* 54:177–196
- Eberhard WG (1990) Imprecision in the behavior of *Leptomorphus* sp. (Diptera, Mycetophilidae) and the evolutionary origin of new behavior patterns. *J Insect Behav* 3:327–357

- Eickwort GC, Ginsberg HS (1980) Foraging and mating behavior in Apoidea. *Annu Rev Entomol* 25:421-446
- Engels E, Engels W (1988) Age-dependent attractiveness for drones and mating in the stingless bee, *Scaptotrigona postica*. *J Apic Res* 27:2-8
- Evans HE, O'Neill KM (1986) Reproductive and nesting biology of *Bembecinus nasus strenuus* (Mickel) (Hymenoptera, Sphecidae). *Proc Entomol Soc Wash* 88:628-633
- Evans HE, O'Neill KM (1988) The natural history and behavior of North American bees. Cornell Univ Press, Ithaca
- Fletcher DJC, Michener CD (eds) (1987) Kin recognition in animals. John Wiley & Sons, Chichester
- Fowler K, Partridge L (1989) A cost of mating in fruitflies. *Nature* 338:760-761
- Free JB (1987) Pheromones of social bees. Cornell Univ Press, Ithaca
- Frisch K von (1967) The dance language and orientation in bees. Harvard Univ Press, Cambridge
- Frohlich DR, Parker FD (1985) Observations of the nest-building and reproductive behavior of a resin-gathering bee: *Dianthidium ulkei* (Hymenoptera: Megachilidae). *Ann Entomol Soc Am* 78:804-810
- Gadagkar R, Bhagavan S, Malpe R, Vinutha C (1990) On confirming the evidence for pre-imaginal caste bias in a primitively eusocial wasp. *Proc Indian Acad Sci (Anim Sci)* 99:141-150
- Greenberg L (1982) Persistent habituation to female odor by male sweat bees *Lasioglossum zephyrum* (Hymenoptera: Halictidae). *J Kansas Entomol Soc* 55:525-531
- Greenberg L (1988) Kin recognition in the sweat bee *Lasioglossum zephyrum*. *Behav Genet* 18:425-438
- Gromko MH, Sheehan K, Richmond RC (1980) Random mating in two species of *Drosophila*. *Am Nat* 115:467-479
- Hirsch J, Holliday M (1988) A fundamental distinction in the analysis and interpretation of behavior. *J Comp Psychol* 102:372-377
- Hölldobler B, Lindauer M (eds) (1985) Behavioral ecology and sociobiology. *Fort Zool* 31:1-488
- Hölldobler B, Michener CD (1980) Mechanisms of identification and discrimination in social Hymenoptera. In: Markl H (ed) *Evolution of social behavior: hypotheses and empirical tests*. Verlag Chemie GmbH, Weinheim, pp 35-58
- Jander J (1968) Über die Ethometrie von Schlüsselreizen, die Theorie der telotaktischen Wahlhandlung und das Potenzprinzip der terminalen Cumulation bei Arthropoden. *Z Vergl Physiol* 59:319-356
- Jacobs W (1924) Das Duftorgan von *Apis mellifera* und ähnliche Hautdrüsenorgane sozialer und solitärer Apiden. *Z Morph Ökol Tiere* 3:1-80
- Keeping MG, Lipschitz D, Crewe RM (1986) Chemical mate recognition and release of male sexual behavior in polybiine wasps, *Belanogaster petiolata* (Degeer) (Hymenoptera: Vespidae). *J Chem Ecol* 12:773-779
- Koeniger G (1990) The role of the mating sign in honey bees, *Apis mellifera* L.: does it hinder or promote multiple mating? *Anim Behav* 39:444-449
- Kukuk P (1985) Evidence for an antiaphrodisiac in the sweat bee *Lasioglossum (Dialictus) zephyrum*. *Science* 227:656-657
- Kukuk P (1989) Dispersal of males of the primitively eusocial sweat bee *Dialictus zephyrus* within a small nest aggregation. *Sociobiology* 15:1-7
- Kullenberg B (1973) Field experiments with chemical sexual attractants on aculeate Hymenoptera males. II. *Zoon (suppl)* 1:32-44
- Larsson F (1989) Mating patterns in six insect species: effects of weather and population density. *Acta Univ Ups (Uppsala)* 234:1-33
- Linsley EG (1958) The ecology of solitary bees. *Hilgardia* 27:453-599
- Lloyd JE (1980) Sexual selection: individuality, identification, and recognition in a bumblebee and other insects. *Fla Entomol* 64:89-118
- Low BS (1978) Environmental uncertainty and the parental strategies of marsupials and placentals. *Am Nat* 112:197-213
- Lynch M (1991) The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution* 45:622-629
- Meyer-Holzappel M (1984) Zur Ethologie des Männchens der Trugbiene (*Panurgus banksianus* Kirby) (Hymenoptera, Apidae). *Z Tierpsychol* 64:221-252
- Michener CD (1982) Early stages in insect social evolution: individual and family odor differences and their functions. *Bull Entomol Soc Amer* 28:7-11
- Michener CD, Smith BH (1987) Kin recognition in primitively eusocial insects. In: Fletcher DJC, Michener CD (eds) *Kin recognition in animals*. John Wiley & Sons, Chichester, pp 209-242
- Michener CD, Wille A (1961) The bionomics of a primitively social bee, *Lasioglossum inconspicuum*. *Univ Kansas Sci Bull* 42:1123-1202
- Miller GA, Galanter E, Pribram KH (1960) Plans and the structure of behavior. Henry Holt and Co, New York
- Minckley RL, Buchmann SL, Wcislo WT (1991) Bioassay evidence for a sex attractant pheromone in the large carpenter bee, *Xylocopa varipuncta* (Anthophoridae: Hymenoptera). *J Zool London* 224:285-291
- Ono M, Sasaki M (1987) Sex pheromones and their cross-activities in six Japanese sympatric species of the genus *Vespa*. *Insectes Soc* 34:252-260
- Packer L (1986) The biology of a subtropical population of *Halictus ligatus* Say (Hymenoptera: Halictidae). II. Male behaviour. *Ethology* 72:287-298
- Peeke HVS, Herz MJ (eds) (1973) Habituation. vol 1, Behavioral studies. Academic Press, New York
- Peeke HVS, Petrinovich L (eds) (1984) Habituation, sensitization, and behavior. Academic Press, New York
- Ratnieks FL (1991) The evolution of genetic odor-cue diversity in social Hymenoptera. *Am Nat* 137:202-226
- Robacker DC, Weaver KM, Hendry LB (1976) Sexual communication and associative learning in the parasitic wasp *Itoplectis conquisitor* (Say). *J Chem Ecol* 2:39-48
- Robson GC, Richards OW (1936) The variation of animals in nature. Longmans, Green & Co
- Serradilla JM, Ayala FJ (1983) Alloproctric selection: a mode of natural selection promoting polymorphism. *Proc Natl Acad Sci USA* 80:2022-2025
- Shimron O, Hefetz A (1985) Mating behavior and sex attraction of *Eucera palestinae* Friese (Hymenoptera: Anthophoridae). *J Kansas Entomol Soc* 58:526-531
- Smith BH (1983) Recognition of female kin by male bees through olfactory signals. *Proc Natl Acad Sci USA* 80:4551-4553
- Smith BH, Ayasse M (1987) Kin-based male mating preferences in two species of halictine bee. *Behav Ecol Sociobiol* 20:313-318
- Sokal RR, Rohlf FJ (1981) Biometry, 2nd edn. W.H. Freeman & Co, New York
- Spies EB, Carson HL (1981) Sexual selection in *Drosophila silvestris* of Hawaii. *Proc Natl Acad Sci USA* 78:3088-3092
- Tengö J (1979) Odour-released behaviour in *Andrena* male bees (Apoidea, Hymenoptera) *Zoon* 7:15-48
- Tengö J, Bergström G (1977) Comparative analyses of complex secretions from heads of *Andrena* bees (Hym., Apoidea). *Comp Biochem Physiol* 57:197-202
- Tengö J, Eriksson J, Borg-Karlson A-K, Smith BH, Dobson H (1988) Mate-locating strategies and multimodal communication in male mating behavior of *Panurgus banksianus* and *P. calcareatus* (Apoidea, Andrenidae). *J Kansas Entomol Soc* 61:388-395
- Thornhill R, Alcock J (1983) The evolution of insect mating systems. Harvard Univ Press, Cambridge
- Thorpe WH (1963) Learning and instinct in animals. Harvard Univ Press, Cambridge
- Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed) *Sexual selection and the descent of man 1871-1971*. Aldine, Chicago, pp 136-179

- Wade MJ (1987) Measuring sexual selection. In: Bradbury JW, Andersson MA (eds) Sexual selection: testing the alternatives. Springer-Verlag, Berlin, pp 197–207
- Wade MJ, Pruett-Jones SG (1990) Female copying increases the variance in male mating success. *Proc Natl Acad Sci USA* 87:5749–5753
- Wcislo WT (1987) The role of learning in the mating biology of a sweat bee, *Lasioglossum zephyrum* (Hymenoptera: Halictidae). *Behav Ecol Sociobiol* 20:179–185
- Wcislo WT (1989) Behavioral environments and evolutionary change. *Annu Rev Ecol Syst* 20:137–169
- Wcislo WT (1990) A new species of *Lasioglossum* from Costa Rica. *J Kansas Entomol Soc* 60:450–453
- Wcislo WT (1991) Natural history, learning, and social behavior in solitary sweat bees (Hymenoptera: Halictidae). Ph.D Dissertation, Univ Kansas, Lawrence
- Wcislo WT (in press) Nest localization and recognition by a solitary sweat bee *Lasioglossum (Dialictus) figueresi* Wcislo (Hymenoptera: Halictidae). *Ethology*
- Wcislo WT, Minckley RL, Spangler HG (in press a). Pre-copulatory courtship behavior in a solitary bee, *Nomia triangulifera* Vachal (Hymenoptera: Halictidae). *Apidologie*
- Wcislo WT, Wille A, Orozco E (in press b). Nesting biology of solitary and social sweat bees, *Lasioglossum (Dialictus) figueresi* Wcislo and *L. (D.) aeneiventre* (Friese) (Hymenoptera: Halictidae). *Insectes Soc*
- West-Eberhard MJ (1983) Sexual selection, social competition, and speciation. *Q Rev Biol* 58:155–183
- Williams RJ (1959) *Biochemical individuality*. Academic Press, New York
- Zawistowski S (1988) A replication demonstrating reduced courtship of *Drosophila melanogaster* by associative learning. *J Comp Psychol* 102:174–176
- Zawistowski S, Richmond RC (1985) Experience-mediated courtship reduction and competition for mates by male *Drosophila melanogaster*. *Behav Genet* 15:561–569