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MATING BEHAVIOR IN HALICTINE BEES (HYMENOPTERA : HALICTIDAE) :

III. COPULATORY BEHAVIOR AND OLFACTORY COMMUNICATION (*)

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SUMMARY

Of the eight species of halictine bees whose mating behavior was studied, *Augochlora pura* (Augochlorini) and *Lasioglossum zephyrum* (Halictini) were investigated in the greatest detail. Halictine bees have a short, simple courtship. Copulation occurs in the male-above position and its duration, 4 to about 340 seconds, varies significantly among species. Females resisted most mating attempts of males and usually mated only once. Males inseminated more than one female.

Males discriminated between conspecific females and those of other species; they did not make significantly more contacts with living, mobile females than with immobile ones killed by freezing. Female odors of *L. zephyrum* and *A. pura* include aphrodisiacs; at least the former has a sex attractant which may be the same compound as the aphrodisiac. Virgin females of *L. zephyrum* 2 to 8 days old produce aphrodisiac; it may be present throughout life. Some halictine females were highly attractive to males for up to 15 days, even after the females had mated. Aphrodisiac caused males of *L. zephyrum* and *A. pura* to pounce upon 3 mm black India ink dots, which they probably mistook for females. Heads, mesosomas, and metasomas of females of *L. zephyrum* all bore aphrodisiac.

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ZUSAMMENFASSUNG

Paarungsbetragen in Halictusbien III. Begatungsverhalten und Geruchsmitteiling

Von den acht Halictusarten, deren Paarungsverhalten studiert wurde, sind *Augochlora pura* (Auglochlorini) und *Lasioglossum zephyrum* (Halictini) am genauesten untersucht worden. Halictusbienen haben eine einfache, kurze Balz. Bei der Kopulation reiten die Männchen auf, und die Kopulationsdauer (4 bis ungefähr 340 sec) ist signifikant unterschiedlich bei den einzelnen Arten. Die Weibchen widersetzen sich den meisten Paarungsversuchen der Männchen und kopulieren gewöhnlich nur einmal. Die Männchen begatten mehr als ein Weibchen.

Die Männchen unterschieden arteigene Weibchen von solchen anderer Arten. Sie nahmen nicht signifikant mehr Kontakt auf mit lebenden Weibchen als mit unbeweglichen, die durch Einfrieren getötet waren. Duftstoff der Weibchen von *L. zephyrum* und *A. pura* enthält Aphrodisiakum; zumindest *L. zephyrum* hat einen Sexuallockstoff, der wahrscheinlich mit dem Aphrodisiakum identisch ist. Zwei bis drei Tage alte, unbegattete Weibchen dieser Art produzieren ein Aphrodisiakum, welches vermutlich während der gesamten Lebensspanne vorhanden ist. Einige weibliche Halictinae waren bis zu 15 Tagen hoch attraktiv für die Männchen, sogar nachdem die Weibchen schon kopuliert hatten. Das Aphrodisiakum veranlasste Männchen von *L. zephyrum* und *A. pura* auf 3 mm grosse, schwarze Tuschepunkte zu springen, die sie wohl für Weibchen hielten. Die Duftstoffe aus den Köpfen, Meso- umd Metasomas von weiblichen *L. zephyrum* enthielten alle das Aphrodisiakum.

INTRODUCTION

This paper concerns the courtship, copulatory, and postcopulatory behavior of halictine bees. Interactions between sexes are aspects of social behavior that have previously been neglected in studies of these bees. In 1972, SAKAGAMI and FUKUDA summarized the scanty literature concerning mating behavior in the Halictinae. Courtship is defined here as behavior of the male and female, subsequent to the male's discovery of the female and prior to insemination, which leads to copulation.

The following is a list of the halictine bees studied with references to principal behavioral papers on them when such papers exist : Tribe Halictini : *Agapostemon splendens* (ROBERTS, 1969), *Lasioglossum (Dialictus) coeruleum** (STOCKHAMMER, 1967), *L. (D.) imitatum** (MICHENER and WILLE, 1961), *L. (D.) laevissimum* (1), *L. (D.) rohweri** (BREED, 1975), *L. (Evylaeus) truncatum and L. (D.) zephryum** (BATRA, 1966; MICHENER *et al.*, 1971 *a*, 1971 *b;* MICHENER and

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(1) Identified as near *Lasioglossum laevissimum* by G. C. EICKWORT. Voucher specimens have been deposited in the Snow Entomological Museum, University of Kansas, Lawrence, Kansas.

BROTHERS, 1974; BROTHERS and MICHENER, 1974; KAMM, 1974; BELL and HAWKINS, 1974; BELL *et al.*, 1974; BELL, 1974). Tribe Augochlorini : *Augochlora pura* (STOCKHAMMER, 1966), and *Augochlorella striata** and *A. persimilis** (ORDWAY, 1966). *Augochlora pura and L. zephyrum* were investigated more than other species because they are representatives of different tribes, their natural histories are already well-known, and they are readily obtainable near Lawrence, Kansas. An asterisk indicates a species known to be social.

MATERIALS AND METHODS

Bees were observed in the field (Douglas and Jefferson Counties, Kansas), in the bee room described by STOCKHAMMER (1966) and BATRA (1966), and in three similar smaller rooms. In the field I observed bees both at nesting sites and around flowers.

The laboratory bee rooms had long day photoperiods (15 hours light) and temperatures from 23 to 37 °C. Flowers, mostly wildflowers, provided nectar and pollen for bees. Flowers used were those preferred by the bees and available in numbers near Lawrence, Kansas. *Apis* honey (diluted with 50 percent water) and cat tail *(Typha)* pollen (kept frozen until used) alone or mixed about half and half with pollen substitute (soy flour), supplemented the food from fresh flowers. Males in cages were fed honey-water each morning; experiments were made with them in afternoons.

Virgin bees of known ages were reared from pupae taken in the field or from bee room nests. The recently emerged bees were harvested from groups of pupae every 24 hours. Since adults emerge at all times of day, a group of « 1-day-old » bees was composed of bees 0 to almost 24 hours old; « 2-day-old » bees, 24 to almost 48 hours old; etc. For individual recognition, 2-day-old bees were marked on their scuta with fast-drying enamel paints. Such bees and others were free in rooms or in cages in the rooms.

Many mating experiments were made with groups of 10 to 30 conspecific males that flew in cages or in the bee rooms. It was impractical to know the ages of all such bees. To assure repeatability, a diverse age structure was maintained by periodically adding both 2-day-old males reared from pupae and wild males to the group of males. Wild ones placed in cages were allowed to acclimate to laboratory conditions for several days before they were used in experiments. It was sometimes necessary to make several pilot tests with caged males before making experiments since the males seemed to learn to find females or odoriferous papers in certain places in the cage before they became sufficiently responsive for experimentation. This suggests that learning of the physical and olfactory features, or probably both, of rendez-vous places (see BARROWS, 1975 *a*, 1975 *d*) is a likely prelude to mating attempts at those places.

Three types of cages were used in the bee rooms. The small cages were cylindrical, 23 cm in diameter × 38 cm high, and made of a wooden frame, netting, and clear plastic. The medium-sized cages, about 0.3 × 1.0 × 1.3 m, had a top and front of clear plastic, sides and back of netting, and bottom of canvas. A zipper down one side permitted access. The back was fastened to a wall of the bee room and the front was supported by strings attached to the ceiling. The large cages were similar to the medium-sized ones, but larger, 1.3 × 1.7 × 1.0 m, only the top was plastic, and they were supported on aluminum frames.

Bee nests were maintained in the laboratory to supply immature bees and so that possible orientation of males around nests could be observed. The wood-inhabiting bees, *L. coeruleum* and *A. pura*, nested in rotting logs and branches of *Ulmus*, in observation nests containing cellulose (described by MAY and STOCKHAMMER, 1968), or sometimes in soil (BARROWS, 1973). The other bees, which were soil-inhabiting, nested in a wooden box or in plastic buckets of soil. Several large lumps of soil containing nests of *L. imitatum*, *L. zephyrum*, *L. rohweri*, and *Augochlorella striata* were excavated from field sites and brought in buckets into bee rooms. Nesting substrates were moistened as needed.

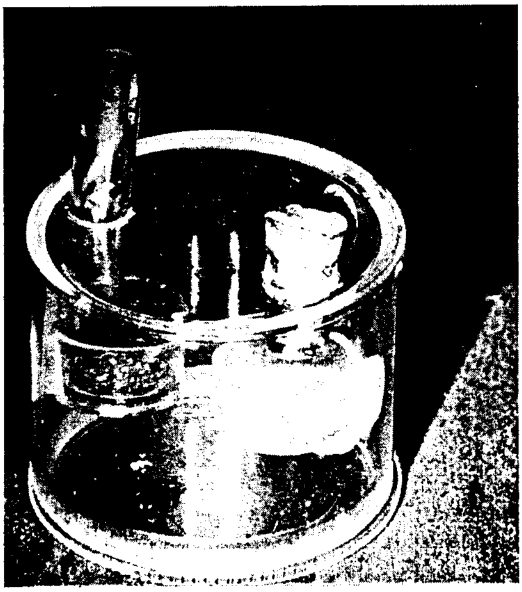

FIG. 1. — Plastic container in which a tethered female bee was imprisioned between test periods. See Materials and Methods.

ABB. 1. — Plastikbehälter zum Aufbewahren der fixierten weiblichen Biene außerhalb der Testperioden.

Some of the females used in experiments involving mating were tethered with human hair tied around their « waists », since untethered females were often difficult to observe or handle. Tethers, 0.5 to 1.5 cm long, did not prevent bees from walking or flying. When she was not being used in an experiment, each tethered female was isolated in a small cylindrical, clear plastic container with honey-water and pollen (fig. 1). A black plastic tube, 4 mm in inside diameter and about 5 cm long, stuck out of the container and was closed at its distal end with a piece of pipe cleaner. The imprisioned bee often rested in this tube. In some experiments a tethered female was presented to males on a white vinyl plate, 5 × 12 cm, to which her tether was fastened with cellophane tape. The plate was attached by a thumbtack to vegetation, a card with dots (see below), or a wall. In experiments involving medium-sized cages of males, the plate was placed inside on the back of the cages about 15 cm below the top.

At the onset of and during certain parts of this study, observations of presumed mating of females were verified by dissecting them. After mating such bees were placed along with moist paper in closed jars or in small cages. About 24 hours later (to allow passage of spermatozoa into the spermathecae) they were fixed in Dietrich's (Kahle's) solution for subsequent dissection and examination of the spermatheca.

To investigate male response toward odors of females, pieces of filter paper (15 cm^2) were moistened slightly with tap water before being impregnated with the odor. Twelve to 16 pieces of paper and female bees (mated and virgin, of various ages) were placed in 237 ml jars closed with corks. Two females of *L. zephyrum* or one female of *A. pura* were included per piece of paper. Control papers were prepared by placing them in similar jars containing no bees. Paper remained in these jars in the dark at 23 to 37 °C for 15 to 24 hours during which time bees were not fed. Except as otherwise noted, all impregnated papers used in a given test were from the same jar and therefore would be expected to have odors of the same group of females. The term « test paper » refers to a filter paper impregnated with female bee odor.

Each test or experiment was composed of trials which were repetitions of a particular experimental procedure.

White cards (21 × 28 cm) with eight dots of black India ink 3 mm in diameter were used in certain experiments (fig. 2). An experiment was made comparing such dots with 12 mm ones but no difference in response of male bees to the two sizes could be discerned. An impregnated paper, control paper, tethered bee, or alone (control) tether was affixed among the dots with cellophane tape (fig. 3). The same dot card and piece of tape were used for all trials of a given test. The rate of male pouncing upon ink dots was recorded. When a male struck a dot, a pounce was recorded unless another male rested or walked within 3 cm of this dot; under these circumstances the pounce was ignored because of the possibility that it was directed toward the other male.

Some terminology used in recording the behavior of halictine males is as follows. An « approach » of a male toward a female was recorded if he flew or crawled within 5 cm of her. A « pounce » was talleyed if he quickly walked or flew toward her, attempted to hold her, or held her for less than 1 second, and then moved away. A « grasp » was recorded if a male held a female for over 1 second. A « mount » is a type of grasp—the male-above mating position typical of Hymenoptera. A « mating attempt » was noted if a male grasped a female and displayed one or more of the following behavioral patterns : tapping his metasoma against hers (up to 10 times in *L. zephyrum*), twisting the tip of his metasoma to the tip of hers, and extending and withdrawing his genitalia.

Copulation (described in more detail below under Copulatory Behavior) was recognized when a male mounted a female, held the tip of this metasoma to the tip of hers, and contracted his metasoma (and sometimes simultaneously flicked his wings, antennae, or both from 1 to 3 times per second. Dissections showed that insemination occurred in most such cases.

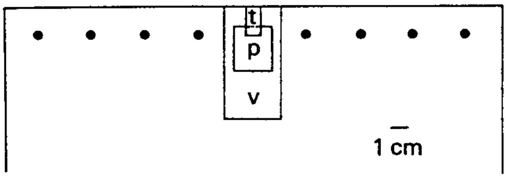

Fig. 2. — White card with black India ink dots. Cellophane tape *(t)* held impregnated or control filter paper *(p)* to white vinyl *(v)* affixed to the card.

Abb. 2. — Weißer Karton mit schwarzen Tuschepunkten. Beduftetes- oder Kontrollfilterpapier *(p)* ist mit Zellophanfilm *(t)* an weißer Kunststoffolie *(v)* befestigt, die an den Karton geheftet ist.

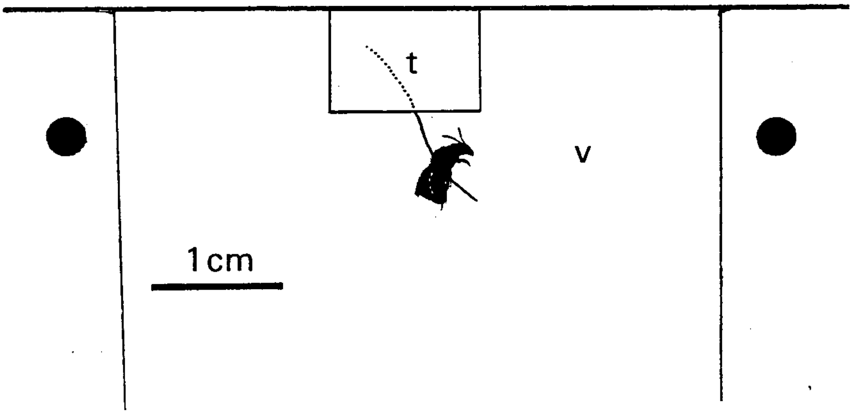

Fig. 3. — Female of *Lasioglossum zephyrum* with her tether fastened with cellophane tape to a dot card. Abbreviations are those used in figure 2.

Abb. 3. — *Lasioglossum zephyrum* Weibchen mit ihrer Halteschlinge an einer punktierten Karte befestigt. Abkürzungen sind die gleichen wie in Abb. 2.

The abbreviation WT is used for Wilcoxon's signed rank test; FT, Fisher's exact probability test; MWT, Mann-Whitney U-test; and M, for the median of a sample. In some experiments probabilities from independent statistical tests were combined using the method of Sokal and Rohlf (1969, p. 623).

PRECOPULATORY BEHAVIOR

Except for territorial males of *Lasioglossum rohweri* (Barrows, 1975 *a*, 1975 *b*) the males studied ordinarily are patrolling (terminology of Barrows, 1974 c, 1975 *a*) when they find mates. After males discover conspecific females, they move towards them, hover over them, pounce upon them, grasp them, attempt to inseminate them, actually inseminate them, separate, and fly away. The sequence of these events often differs from one encounter of a male with a female to the next (fig. 4); relative frequencies of different sequences have not yet been studied.

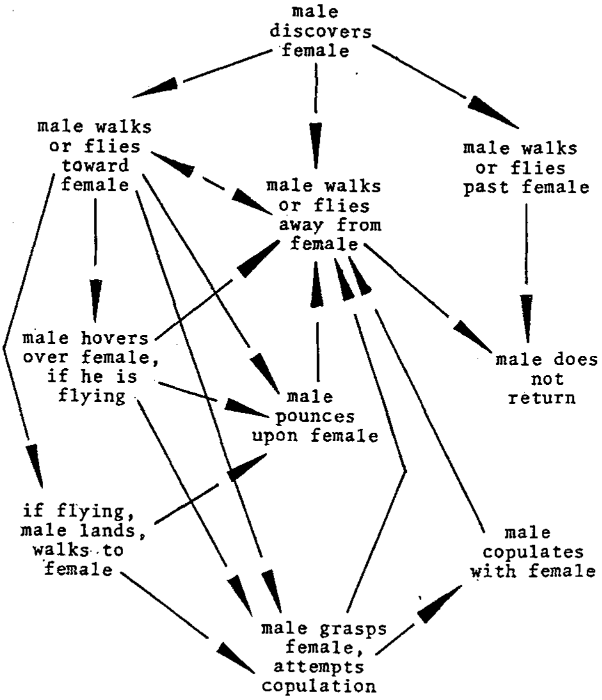

Fig. 4. — Diagram illustrating the diverse sequences of responses of a male halictine bee toward a female after discovery of her. The arrows indicate possible sequences of responses.

Abb. 4. — Pfeildiagramm zur Veranschaulichung der möglichen Reaktionsfolgen einer männlichen Halictus-Biene gegenüber einem Weibchen, nach dessen Entdeckung.

Airbone males respond toward females in various ways. They often quickly fly from side to side over them or hover over them (fig. 5). Sometimes males of *L. rohweri* and *A. pura* repeatedly fly towards females, hover close to them, fly 2 to 3 cm from them, and fly toward them again (fig. 6). Males of *L. zephyrum* often pounce upon or grasp females without first hovering near them. In contrast males of *A. pura* almost always hover near females before pouncing upon or grasping them. As males fly near females they often touch them with the tips of their antennae. Besides conspecific females, males are attracted to objects including holes and black spots which are similar in size to females (BARROWS, 1975 *a*, 1975 *d*).

Based on observations with tethered females, durations of grasping of females (excluding copulation) by males of *L. zephyrum* vary from 1 to 110 seconds (median = 2 seconds, n = 482 observations); those of *A. pura*, from 1 to 97 seconds (2, 526); and those of *L. rohweri*, from 1 to 172 seconds (8, 197). Median duration of grasping females by males varied significantly among species ($\chi^2 = 55.197$, $df = 2$, $p < .001$, $n = 1001$, median test).

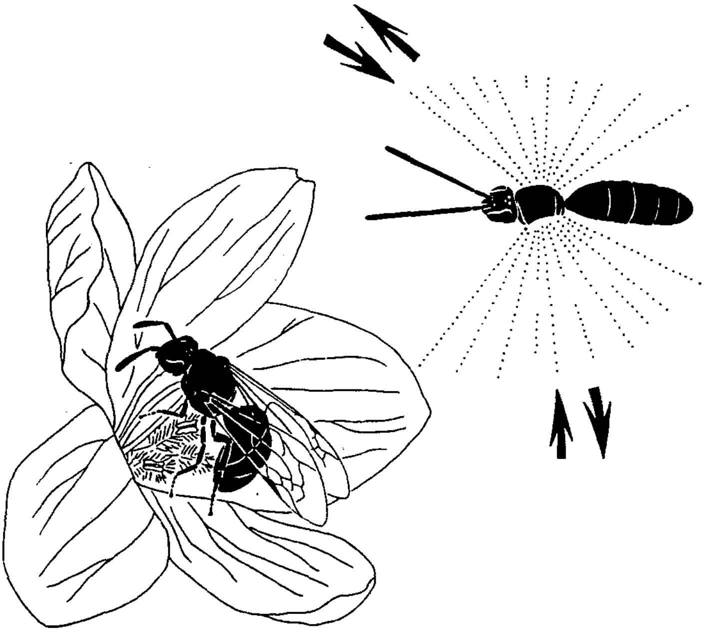

FIG. 5. — Side to side flight of a male halictine bee over a female on a flower.

ABB. 5. — Zick-Zack-Flug eines Halictus Männchens über einem Weibchen auf einer Blume.

In halictine bees the male preparing to copulate takes a horizontal position over the female on the female's dorsum, the tip of his metasoma curled over that of the female. If tethered females are suspended so that their dorsums are against substrates, the males ordinarily land on females' exposed surfaces and quickly crawl between them and the substrate to attain the usual mating position. Not infrequently up to four males of *Augochlora pura*, *Augochlorella striata*, or *L. zephyrum* stack themselves atop a male on a tethered conspecific female. In this situation males attempts mating with one another and those close to the female all try to copulate with her. Sometimes up to six other males crawl over mating pairs. Similar behavior occurs in the field when males of *L. rohweri* cluster around the same female (BARROWS, 1975 *a*, 1975 *b*). The original male of the pair either flies away or seems to ignore the intruders. Once males of *A. pura* grasp females, they are unwary and have to be pulled off to prevent insemination.

Occasionally males, possibly inexperienced ones, do not immediately attain the usual mating position after contacting females but try to copulate with females' heads or walk over females in a frenzy. Usually such males eventually assume the typical male-above position.

Courtship behavior in halictine bees is simple and brief. Males were observed to mount females for up to 5 seconds before initiation of copulation and there was no obvious courtship behavior prior to mounting. Various stimuli (i.e., vibration of substrates on which females rest caused by beating wings, alighting movements, and other movements of males; and tactile stimuli caused by males grasping females, curling tips of metasomas to those of females, tapping metasomas against those of females, and as seen only in *A. pura,* repeatedly stroking heads of grasped females with antennae) may prepare females for copulation. It is possible that visual stimuli produced by females which go to rendez-vous places (Barrows, 1975 *a,* 1975 *b*) and, as described below for *L. zephyrum,* bearing sex attractant and aphrodisiac by such females, are all important in causing males to copulate.

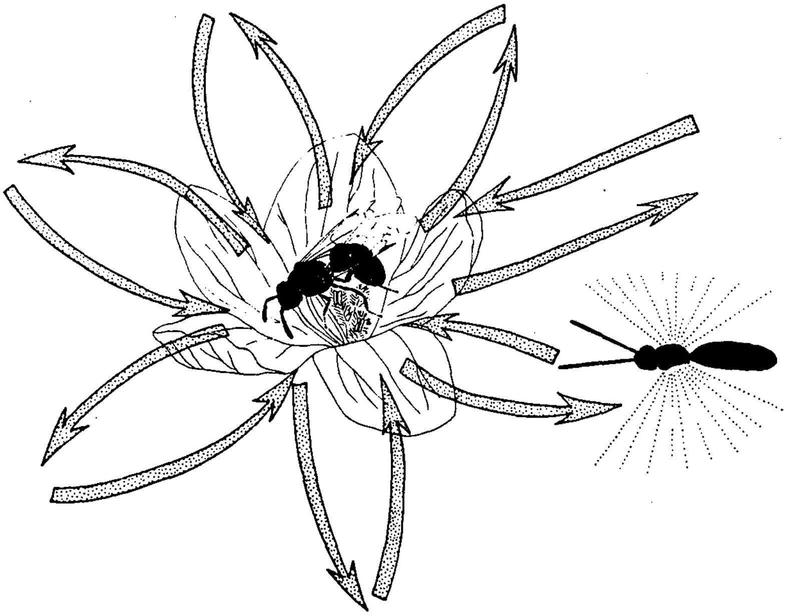

Fig. 6. — The flight of a male halictine bee around a conspecific female on a flower. See text for details.

Abb. 6. — Flug einer männlichen Halictus-Biene um ein auf einer Blume sitzendes arteigenes Weibchen. Einzelheiten im Text.

COPULATORY BEHAVIOR

A mounting male usually clings to the female's metasoma with his legs (fig. 7, *A-C*); less commonly the forelegs cling to the mesosoma or the male touches the substrate with 1 to 4 legs while holding the female with the remaining legs. Males of *A. pura* sometimes release their grasps of females after copulation begins and remain joined to them only by genitalia. Males of *L. zephyrum* hold their antennae together over their dorsums or directed forward, not touching females with them during coition (fig. 7, *A* and *C*). In contrast, males of *A. pura* sometimes repeatedly touch heads of females (up to 8 times) with their antennae while *in copulo.*

Table 1 shows data concerning the duration of copulation in halictine bees. In the laboratory, it varied significantly among all species studied ($\chi^2 = 35$, df $= 3$, $p < .001$, $n = 44$, median test); moreover, in *L. zephyrum* duration of copulation of tethered females in the laboratory was significantly longer than in the field ($p < .001$, MWT), perhaps because of temperature differences and disturbances by breezes, other males, etc.

TABLE I. — Duration of copulation. Data are based on matings of tethered females and free-flying males except for *Lasioglossum laevissimum* in which the bees were free and the time of beginning of mating was not observed.

TABELLE I. — Kopulationsdauer. Die Daten beziehen sich auf Paarungen von fixierten Weibchen und frei fliegenden Männchen. Nur bei *Lasioglossum laevissimum* waren die Bienen frei und der Zeitpunkt des Paarungsbeginns wurde nicht beobachtet.

Species	Duration (seconds)		
	Range	Median	n
Lasioglossum zephyrum (field)	10-42	14	12
L. zephyrum (laboratory)	31-70	48	12
L. laevissimum (laboratory)	96(+)-340(+)	202.5(+)	6
L. rohweri (laboratory)	4-10	8	16
Augochlora pura (field)	23-34	28.5	2
A. pura (laboratory)	17-56	28	10

During copulation males of *A. pura* and of *L. zephyrum* contract their metasomas about once per second; those of *L. rohweri,* 1 to 3 times per second. Sometimes males twitch wings, antennae, or both simultaneously when contracting metasomas.

Tethered females of *L. coeruleum, laevissimum, rohweri, truncatum,* and *zephyrum* are often motionless during copulation or they slowly move their heads, antennae, or legs, but do not crawl. Tethered females of *A. pura* often crawl slowly on vegetation and occasionally bite males' metasomas while *in copulo.*

Fig. 7. — *Lasioglossum zephyrum in copulo.* The male has a light-colored face and longer antennae than the female which is tethered.

Abb. 7. — *Lasioglossum zephyrum* bei der Kopulation. Das Männchen hat ein helleres Gesicht und längere Antennen als das Weibchen, das festgebunden ist.

Near the end of copulation females occasionally buzz with their wings folded over their backs; males terminate copulation by withdrawing genitalia before or when they start flying. In one instance, at the end of mating a male of *L. rohweri* groomed his hindlegs before flying. Sometimes males stop near the copulating site and groom for about 1 minute before flying. Immediately after copulation, however, most males resume patrolling.

MATING FREQUENCY

Tethered females.

In attempts to investigate mating frequency as well as possible changes in attractiveness with age (described below under Varying Attractiveness of Females), I presented tethered females to flight groups of conspecific males. The females were presented for 3 to 10 minutes, first when they were 2-day-old adults, and subsequently on alternate days until they were 18 days old or until they died. Of a total of 56 matings observed in *A. pura*, *L. rohweri*, and *L. zephyrum*, 96.5 percent occurred during the first 2 minutes of a presentation. Six of seven females of *L. zephyrum* that mated during 10 minute presentations started mating during the first minute and did not mate again in the 10-minute periods. Many presentations, therefore, were made for only 3 minutes.

If they mated at all, tethered halictine females usually mated only once during a series of presentations. Ten such females of *A. pura* (surviving from 6 to 18 days, M = 14) each mated once (at 2 to 6 days old, M = 2). Twenty others (surviving from 8 to 18 days, M = 18) did not mate. Fifteen females of *L. zephyrum* (surviving from 4 to 29 days, M =14) each mated once (at 2 to 14 days, M = 3). Eighteen other tethered females (surviving from 4 to 27 days, M = 12) did not mate. Eleven females of *L. rohweri* (surviving from 2 to 18 days, M = 10) mated; eight of them, only once at the age of 2 days, three of them 2 or 3 times at 2 days of age and later at 4 to 18 days. Twenty-one other females that survived 4 to 18 days (M = 12) did not mate. In other experiments multiple matings of *A. pura* and *L. zephyrum* were noted rarely but such behavior is possibly more common in *L. rohweri*.

All females of these three species used in the experiments on mating frequency were virgins, probably potentially reproductive; therefore, it is unclear why so many refused to mate. In *L. zephyrum* caste determination occurs in the adult stage (BROTHERS and MICHENER, 1974); thus any female can potentially become a queen and in the laboratory nearly all queens are inseminated (MICHENER *et al.*, 1971 *a*). It seems, therefore, that most if not all tethered, virgin females of this species should have mated. *L. rohweri*, like *L. zephyrum*, is a primitively eusocial bee so all of its females might be expected to be potential queens which become inseminated. Moreover, since *A. pura* is a solitary bee, most if not all of its females would be expected to mate. Possible reasons for the halictine females not mating include internal parasitization (for which dissections of population samples provide no evidence) and artificiality of experimental conditions. The higher percentages of mating of old, free females in cages (see next section) appear to show that the tethers or other laboratory conditions have an inhibiting effect on mating. Field observations too indicate a high frequency of refusal to mate by females, but in such cases one cannot be certain that the females are virgin.

Males.

In an experiment that shows that males can inseminate more than one female, 2 or sometimes 3 free-flying, virgin females of *A. pura* or *L. zephyrum,* 2 to 20 days old, were placed in a medium-sized cage with one conspecific male for 72 hours. One active male, which may or may not have been virgin, from a bee room flight group was used in each of nine repetitions of this experiment. All females were later dissected to determine how many had mated. The males inseminated both females in 2 of 7 repetitions using *A. pura* and in the 2 repetitions using *L. zephyrum.*

Ages at mating.

Experiments with free, marked, virgin females were made to further examine conclusions drawn from tests using tethered females as described above. Tethered females usually mated, if at all, when 2 to 4 days old.

Old virgin females of *L. rohweri* seem to mate as readily as young virgin females. Thirty-four females and an equal number of males were placed in a large cage for 24 hours. Seventeen of the females (group 1) were from 2 to 6 days old ($M = 4$) and 17 females (group 2) were from 7 to 14 days old ($M = 8$). Subsequent dissections showed that 77 percent of the females had eaten pollen while in a medium-sized retaining cage and all had slender ovaries. Sixty-five percent of the group-1 females and 59 percent of the group-2 females mated, showing no significant difference between groups ($p = .259$, FT).

Likewise for *L. zephyrum,* old, virgin females seem to mate at least as readily as young ones. Eighty-eight females (marked for individual recognition) and an equal number of males were placed in a medium-sized cage. Mating was infrequent after the first 40 minutes and after 80 minutes, I discontinued observation. Of the 37 females observed to mate, none mated more than once. There were 33 females (group 1) from 3 to 15 days old ($M = 8$ days) and 55 females (group 2) from 16 to 29 days old ($M = 26$). Thirty-nine percent of the group-1 females mated; 44 percent of the group-2 females mated, showing no significant difference between groups ($\chi^2 = .152$, df $= 1$, $p > 0.05$, test for inequality of two percentages, Sokal and Rohlf, 1969).

Also in *A. pura* young and old virgin females did not mate at significantly different frequencies. Eighty females but only 42 males were released in a medium-sized cage and observed for 60 minutes. All mating occurred during the first 20 minutes of observation, suggesting that sexually active bees soon find mates under these experimental conditions. There were 35 females (group 1) from 2 to 16 days old ($M = 13$) and 45 females (group 2) from 17 to 32 days old ($M = 28$). Nine percent of the group-1 females and 11 percent of the group-2 females mated, showing no significant difference between age groups ($\chi^2 = 2.822$, df $= 1$, $p > 0.1$). Of the eight females observed to mate, one mated more than once (see Mating Frequency).

Unlike the females, young males are perhaps more likely to mate than old ones. Twenty of the males of *A. pura* used in the above experiment (group 1) were 2 to 14 days old (M = 9) and 22 males (group 2) were 16 to 30 days old (M = 28). Twenty-five percent of the group-1 males and 5 percent of the group-2 males mated, approaching a significant difference between groups ($p = .065$, FT).

Female resistance to mating.

In nature males of *L. rohweri* and *L. zephyrum* commonly attempt copulation with guards that close nest entrances with their metasomas. They follow females walking to their nests and frequently attack females, including those heavily laden with pollen, that hesitate before entering nests. Males were not observed pursuing females in flight.

In the laboratory both mated and virgin, tethered females often resisted mating attempts by males. Such females strike males with mandibles or legs, buzz their wings, and apparently will not open their genital chambers. Untethered females that resist mating eventually escape by scrambling into burrows or flying. They often bite the metasomas of males which mount them. In the field females of *L. zephyrum* often roll down the nesting banks, thereby usually dislodging males and in the laboratory females fall from walls with the same result. Males of *L. zephyrum* and *L. rohweri* sometimes hold females for over 3 minutes while trying to copulate with them. These observations suggest that the female can prevent mating at will and she might play a major role in selecting her mate.

SEX ATTRACTION AND COPULATORY STIMULATION

Male response toward heterospecific females.

In nature male halictine bees frequently approach and occasionally pounce upon females of other species; this behavior was investigated in more detail in the laboratory. Tests of 7 to 10 trials were made; in each trial a tethered female of one species was presented upon a vinyl plate to a flight group of 20 males of another species for 1 minute and then replaced by a conspecific female, also presented for 1 minute. Different females were used in each trial; pounces, grasps, attempted copulations, and copulations were noted, and for statistical purposes are combined as contacts of males with females. Since males did not approach a given female with equal frequency, it was necessary to standardize contact rate of males with females by measuring male response as contacts per approach.

No heterospecific matings were observed. Males of *L. zephyrum* did not approach conspecific females significantly more than heterospecific ones except in the experiment with *Augochloropsis* (fig. 8, *D*). They occasionally pounced upon females of other species, but infrequently grasped them. Male contact rate

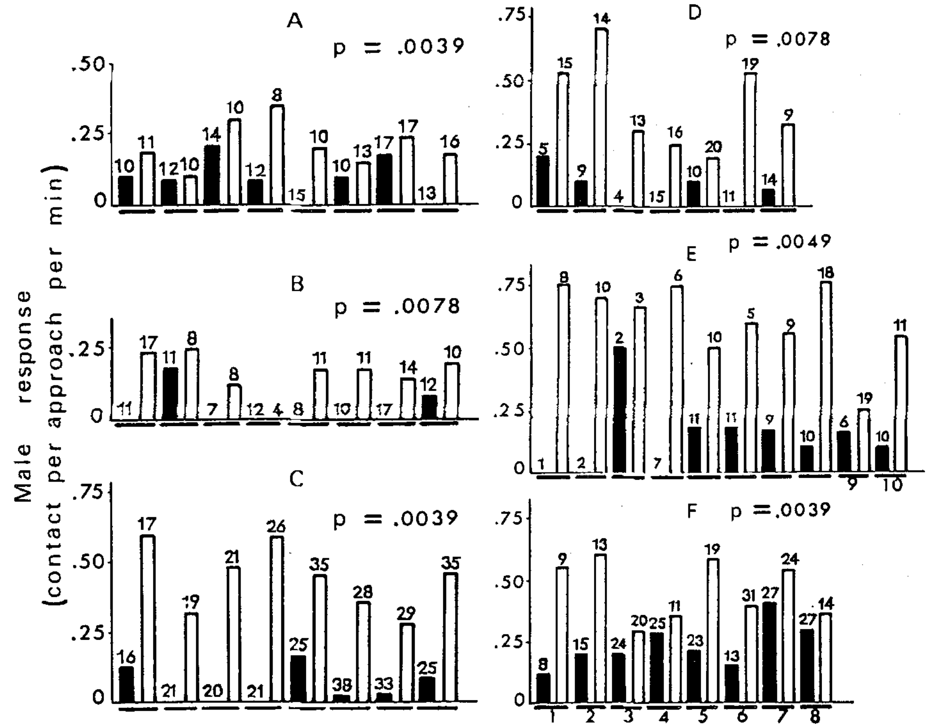

Fig. 8. — Responses of males of *Lasioglossum zephyrum* toward females of other species (solid bars) and toward conspecific females (open bars). Number of male approaches toward a particular female during a 1 minute presentation is indicated above each bar. Successive trials are indicated on the abscissa. *A*, male response towards females of *Augochlorella striata* (females were not grasped) and *L. zephyrum* (females were grasped 0-2 times, M = 1); *B*, *Agapostemon splendens* (0,0) and *L. zephyrum* (0-2, 0); *C*, *Augochlora pura* (0, 0) and *L. zephyrum* (2-7, 4); *D*, *Augochloropsis metallica* (0-1, 0) and *L. zephyrum* (0-5, 2); *E*, *L. rohweri* (0-1, 0) and *L. zephyrum* (2-13, 3); and *F*, *L. imitatum* (0-5, 0) and *L. zephyrum* (1-8, 4). Wilcoxon's signed rank test was used to analyze differences in male response toward heterospecific and conspecific females.

Abb. 8. — Reaktionen von *Lasioglossum zephyrum* Männchen gegenüber Weibchen anderer Arten (schwarze Säulen) und gegenüber arteigenen Weibchen (offene Säulen). Ueber jeder Säule ist die Anzahl der männlichen Annäherungen an ein bestimmtes Weibchen während einer Minute angegeben. Aufeinander folgende Annäherungsversuche sind auf der Abszisse angegeben. *A*, Männliche Reaktion gegenüber Weibchen von *Augochlorella striata* (die Weibchen wurden nicht umfaßt) und *L. zephyrum* (die Weibchen wurden 0-2 mal umfaßt, M = 1); *B*, *Agapostemon splendens* (0, 0) und *L. zephyrum* (0-2, 0); *C*, *Augochlora pura* (0, 0) und *L. zephyrum* (2-7, 4); *D*, *Augochloropsis metallica* (0-1, 0) und *L. zephyrum* (0-5, 2); *E*, *L. rohweri* (0-1, 0) und *L. zephyrum* (2-13, 3); und *F*, *L. imitatum* (0-5, 0) und *L. zephyrum* (1-8, 4). » Wilcoxon's signed rank test « wurde verwendet, um die Unterschiede zwischen artfremden und arteigenen Weibchen zu analysieren.

with conspecific females was significantly higher than that with heterospecific females (fig. 8).

The males of *A. pura* approached conspecific females significantly more than heterospecific ones in the tests made with *Agapostemon* and *Augochlorella* ($p = .005$, $.01 > p > .005$, respectively, WT), but they approached conspecific females significantly less than those of *Lasioglossum* ($.025 > p > .01$). Males of *A. pura*, moreover, contacted conspecific females significantly more than females of other species except in the test with *Augochloropsis* (fig. 9). However, the males grasped only conspecific females and failed even to contact females of *Augochlorella*. *Augochlora pura, Agapostemon splendens, Augochlorella striata,* and *Augochloropsis metallica* are bright, metallic green and the *Lasioglossum* species are dull metallic greenish-brown; the species also vary markedly in size. The results of these experiments suggest that investigation should be made on the possible roles of size and color of females in discrimination by males.

Activities of males in the field suggest that visual cues may not be as

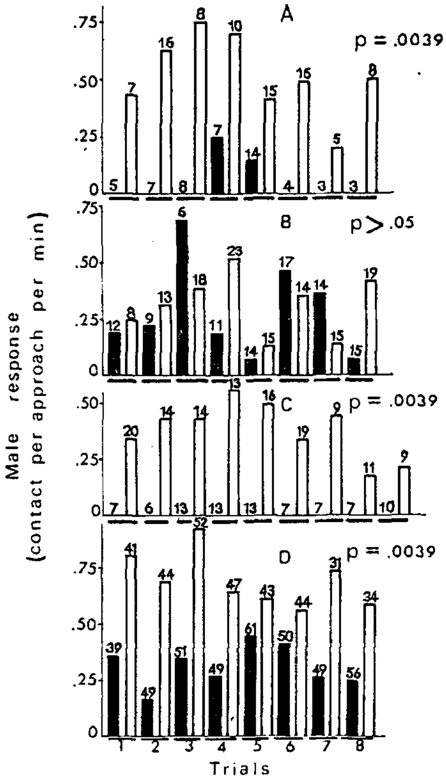

FIG. 9. — Response of males of *Augochlora pura* toward females of other species (solid bars) and toward conspecific females (open bars). Numbers of male approaches toward females during 1 minute presentations are given above bars. *A*, male response towards females of *Agapostemon splendens* (females not grasped) and *A. pura* (females were grasped 0 to 6 times each, M = 2 grasps); *B*, *Augochloropsis metallica* (0, 0) and *A. pura* (0-4, 1); *C*, *Augochlorella striata* (0, 0) and *A. pura* (0-5, 2); and *D*, *Lasioglossum zephyrum* (0, 0) and *A. pura* (0-12, 3). Wilcoxon's signed rank test was used to analyze difference in male response toward heterospecific and conspecific females.

ABB. 9. — Reaktion von *Augochlora pura* Männchen gegenüber artfremden- und arteigenen Weibchen. Ueber den Säulen ist die Anzahl der Annäherung der Männchen zu den Weibchen während einer Minute angegeben. *A*, Reaktion der Männchen gegenüber *Agaspostemon splendens* Weibchen (wurden nicht umfaßt) und *A. pura* (Weibchen wurden je 0-6 mal umfaßt, M = 2; *B*, *Augochloropsis metallica* (0, 0) und *A. pura* (0-4, 1); *C*, *Augochlorella striata* (0, 0) und *A. pura* (0-5, 2); und *D*, *Lasioglossum zephyrum* (0, 0) und *A. pura* (0-12, 3). » Wilcoxon's signed rank test « wurde verwendet, um die Unterschiede zwischen arteigenen und artfremden Weibchen zu analysieren.

important as olfactory ones in distinguishing heterospecific from conspecific females and other female-sized objects. After males approach, they probably recognize conspecific females by species specific pheromones as do *Bombus* males (Apidae) (FREE, 1971).

Effect of females on male dot pouncing behavior.

Since males seemed more inclined to pounce upon spots and shadows on vegetation and upon black ink spots (about the sizes of conspecific females) when conspecific females were nearby than when they were absent, I tried to ascertain if, in fact, the presence of females causes males to pounce upon black dots. Tethered females from the field and unused tethers alone were presented on dot cards (described in Materials and Methods) to flight groups of about 20 males in medium-sized cages. In each trial a tether was presented for 2 minutes on a dot card and then was replaced 30 to 60 seconds later with a tethered female, also presented for 2 minutes. Different tethers and different tethered bees were used in each trial.

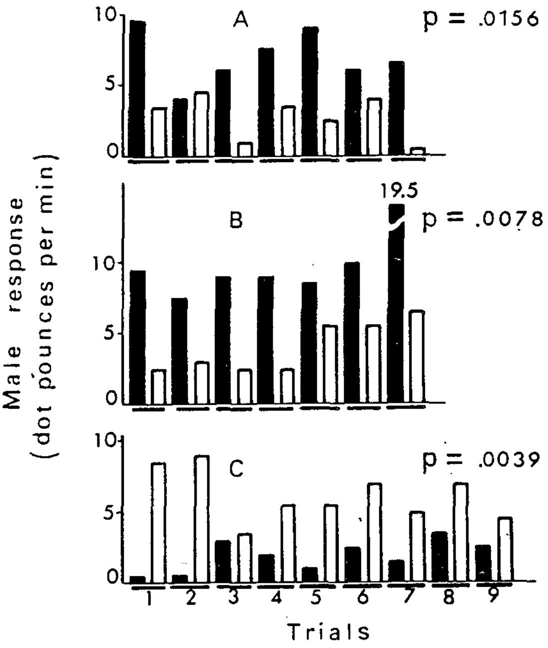

FIG. 10. — Male pouncing on black dots in the presence (open bars) and in the absence of conspecific females (solid bars). *A*, and *B*, *Augochlora pura; C*, *Lasioglossum zephyrum*. Wilcoxon's signed rank test was used to analyze differences in male response in the presence and absence of females.

ABB. 10. — Säulen veranschaulichen die Anzahl der Zustöße der Männchen auf schwarze Punkte in Gegenwart von arteigenen Weibchen (offene Säulen) und in Abwesenheit von arteigenen Weibchen (schwarze Säulen).

In *A. pura,* males pounced upon dots in the presence of females significantly less than in the absence of females (fig. 10 *A* and *B*, $.005 > p > .001$, 2 WT combined). In contrast, males of *L. zephyrum* pounced upon dots significantly more when females were presented among dots than when females were absent (fig. 10 *C*). Males of *A. pura,* unlike those of *L. zephyrum,* directed their attention toward tethered females rather than toward dots. Perhaps males of *A. pura* find females visually among dots more readily than do males of *L. zephyrum.*

It seemed that a female of *A. pura* among dots caused males to pounce upon dots after her removal. To test this hypothesis a dot card was placed in a medium-sized cage with 20 males for 30 to 60 minutes. Then a tether alone (first presentation) was presented on the card to males, then a tethered female, and finally the unused tether (second presentation) was presented again. All presentations were for 2 minutes. On a given day only one trial was made with a particular group of males. Males pounced upon dots significantly more during second presentations of tether than during first ones (fig. 11), thus supporting the hypothesis and the idea that a pheromone left after removal of the female promotes dot pouncing.

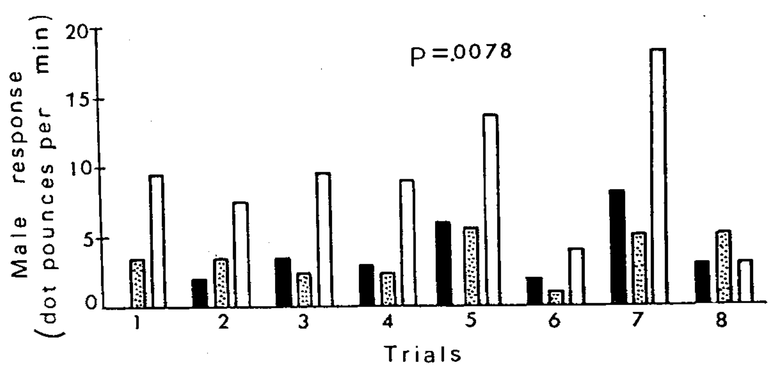

FIG. 11. — Dot pouncing of males of *Augochlora pura* during first presentations of tethers (solid bars), during presentations of females (stipped bars), and during second presentations of tethers (open bars). Dot pounces per minute were calculated from 2-minute observations. Wilcoxon's signed rank test was used to evaluate differences in male response toward first and second presentations of tethers.

ABB. 11. — Zustöße von männlichen *Augochlora pura* auf schwarze Punkte während einer Minute (nach 2 Minuten Beobachtung gemittelt). Schwarze Säulen = erstmalige Darbietung von fixierten Weibchen, punktierte Säulen = Darbietung freier Weibchen, offene Säulen = zweite Darbietung fixierter Weibchen. » Wilcoxon's signed rank test « wurde verwendet, um die Unterschied in der Reaktion der Männchen während der ersten und zweiten Darbietund der fixierten Weibchen zu bewerten.

Varying attractiveness of females.

Females often vary in their attractivity to males, to judge by experiments on Mating Frequency (described above) in which tethered females were presented on alternate days to males. The terminology used in this section is as follows : attractive female, one which males grasp for up to 3 seconds in any of 1 or more presentations; highly attractive female, one which males grasp over 3 seconds while attempting mating, insemination, or both in any presentation; intermittently highly attractive female, one which males find highly attractive in 2 to 4 presentations each of which is between presentations when she was not highly attractive; and consistently highly attractive female, one which is highly attractive during all presentations to males.

From table II it can be seen that different females which mated and which did not mate were attractive, inermittently highly attractive, and consistently highly attractive. Only certain females which did not mate were never highly attractive. Possible reasons for varying attractiveness of females include : some females have sex attractants and some do not; a given female which emits such an attractant may produce differing amounts during her lifetime; and levels of male response are different.

Olfactory cues

Sex attractants. In an attempt to investigate the possible existence of sex attractants, it was found that moist filter paper could be impregnated with odor of females if they were confined with the paper (see Materials and Methods). Nine flight groups of 10 to 20 males of *L. zephyrum* were each presented with a control paper and a test paper (one impregnated with the odor of conspecific females) presented sequentially, each for 3 minutes. The papers were pinned near the ceilings of laboratory rooms where males were patrolling.

Males of *L. zephyrum* responded toward impregnated paper in that they flew toward it, hovered over it, or walked upon and antennated it. The paper was scored as attractive to males if one or more males walked upon and antennated it. Test papers were significantly more attractive than controls ($p = .00003$, $n = 9$, FT), indicating that females of *L. zephyrum* have a sex attractant.

Regarding *A. pura*, a total of 50 presentations of pairs of papers (one test, one control) were made to males in laboratory experiments and in field experiments described by Barrows (1974 *a*). Males usually were not attracted toward either test or control papers possibly because they are more readily attracted to a combination of appropriate olfactory and visual cues than to olfactory ones alone. However, upon six occasions, they walked upon and antennated test papers but not controls, suggesting a sex attractant in this species also. Artificial and natural sex attractants are already known in other species of bees (Kullenberg, 1956, 1963; Butler, 1964; Free, 1971).

Pheromone which effects male dot pouncing behavior. Experiments were made with *A. pura* and *L. zephyrum* to determine whether female odor alone is sufficient to elicit male dot pouncing behavior. In each trial of a test, a control paper was presented on a dot card for 2 minutes and then it was replaced by a test paper impregnated with odor of conspecific females (see Materials and Methods), also presented for 2 minutes. Presentations were made to flight groups of 20 males in medium-sized cages. In *A. pura* presence of female odor (test paper) near dots significantly increased male dot pouncing (fig. 12, $p < .001$, 2 WT

Table II. — Male response toward tethered conspecific females first presented as 2-day-old virgins and thereafter on alternate days until they died or were 18 days old. Ranges and medians of ages of females at their last presentations to males are indicated in parentheses. For definitions of attractive, highly attractive, consistently highly attractive, and intermittently highly attractive, see text.

Tabelle II. — Reaktion von Männchen gegenüber artgleichen Weibchen; zuerst zwei Tage alt unbegattet angeboten, und danach an jedem zweiten Tag, bis sie starben oder 18 Tage alt waren. Das Alter der Weibchen bei ihrem letzten Kontakt mit den Männchen ist in Klammern angegeben. Die Definition für » attractive, highly attractive, consistently highly attractive, and intermittently highly attractive « ist im Text ersichtlich.

	Lasioglossum zephyrum	*Lasioglossum rohweri*	*Augochlora pura*
♀♀ *which mated :*			
intermittently highly attractive	11 (12-18,14)	5 (8-16,12)	6 (14-18,16)
attractive	3 (4-6,5)	4 (2-14,5)	0
consistently highly attractive	3 (6-18,16)	5 (8-18,8)	2 (6,6)
♀♀ *which did not mate :*			
intermittently highly attractive	4 (10-18,14)	10 (8-18,13)	12 (12-18,18)
attractive	5 (6-18,12)	6 (6-14,8)	6 (4-14,9)
consistently highly attractive	0	3 (4-18,14)	0
never highly attractive	9 (4-14,10)	2 (10-14,12)	3 (8-12,10)
Total ♀♀	35	35	29

combined) but in *L. zephyrum* this occurred in only one of four tests in which odor presentations were made for 2 minutes ($p = .05$, $n = 6$, WT).

The sex attractant study suggested that males of *L. zephyrum* « habituate » toward female odor, i.e., show a waning response toward dots in the presence of this odor. Therefore the above dot pouncing experiment was repeated with this species; but to compensate for presumed habituation, presentations of test and control papers were made for only 1 minute each. Presence of female odor significantly increased male dot pouncing in these tests ($.005 > p > .001$, $n = 20$, 2 WT combined).

These tests suggested that males of *A. pura* and *L. zephyrum* habituated to pheromone of conspecific females, they fatigued during tests, or both. Male response (in the presence of test papers) in first halves of tests was greater than that during second halves (*A. pura*, $p = .05$, $n = 8$, WT; *L. zephyrum*, $p < .005$, $n = 20$, WT). Since males did not appear to decrease flying or hovering over dots during test periods, odor habituation seems likely.

Aphrodisiac. In *Augochlora pura* and *Lasioglossum zephyrum* female odor is aphrodisiacal, i.e., it induces copulatory behavior. It caused males of *A. pura*

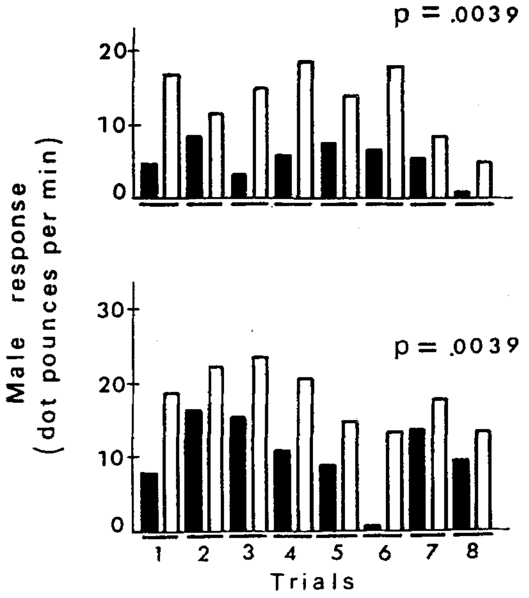

FIG. 12. — *Augochlora pura*. Male dot pouncing in the presence (open bars) and absence (solid bars) of odors on paper from conspecific females. Wilcoxon's signed rank test was used to analyze the differences in male response.

ABB. 12. — *Augochlora pura* Zustöße von Männchen auf schwarze Punkte in Anwesenheit (offene Säulen) und Abwesenheit (schwarze Säulen) von arteigenen Weibchenduft auf Filterpapier. » Wilcoxon's signed rank test « wurde verwendet um die Unterschiede in der Reaktion der Männchen zu bestimmen.

to contact dead conspecific females and to make long contacts (grasps and attempted matings) with them. As mentioned above, in *L. zephyrum,* female odor caused males to pounce upon black ink dots. Since male pouncing upon these dots (and upon other objects about the size of females) appears to be misdirected copulatory behavior, female odor which releases male dot pouncing behavior is considered aphrodisiacal in this study.

The aphrodisiacal odor is probably produced by adult bees although the possibility exists that they somehow acquire it from their habitats. As described below, bees reared in the laboratory (in petri dishes, from field collected pupae) carried aphrodisiacal odor. Pupae from which they emerged shed cuticles upon becoming adults; the latter never contacted nests or usual foods as they would in nature. These aphrodisiacal odors are therefore referred to as aphrodisiacs, i.e., pheromones which induce copulatory behavior.

Female corpses of *A. pura* were presented to males in the presence of either test or control papers. In the first experiment a dead, unextracted female was fastened by her legs, head upward, with cellophane tape to a plate and left 24 hours in a group of 20 males flying in a medium-sized cage. The males paid little attention to her. In each of eight sequential trials of this test, a piece of control paper was taped above the dead female for 1 minute and then replaced for 1 minute with a test paper. Males did not approach the corpse significantly more or less (WT) in the presence of test paper than in the presence of control paper. However, they contacted the corpse significantly more in the presence of test paper ($p = .027$, $n = 8$, WT).

In the second experiment different, dead « deodorized » females were each presented for 1 minute with a control paper and then for 1 minute with a test paper to investigate the possibility that test paper would make these females more attractive to males. The females were collected from the field, killed by freezing, placed in an open dish for 3 days, washed with 70 percent ethanol, acetone, and distilled water, and finally dried for 24 hours at room temperature. Again males did not approach corpses presented with paper significantly more or less than corpses presented with control paper (WT). On the other hand, they contacted the female corpses significantly more in the presence of test papers than in the presence of controls ($p = .004$, $n = 9$, WT).

In both experiments, males of *A. pura* made significantly more long contacts (grasps and attempted matings) with corpses in the presence of odors of conspecific females than in the absence of such odors (first experiment, $p = .020$, $n = 8$, WT; second experiment, $p = .004$, $n = 9$, WT).

Experiments of this kind were not made with *L. zephyrum* because the level of male response toward dead, extracted females in the presence of odor of conspecific females fell to zero after only 3 to 4 presentations, suggesting odor habituation of males.

Aphrodisiacs and artificial aphrodisiacal odors have been previously reported for other bees (KULLENBERG, 1956, 1973; BUTLER, 1967, 1971). Moreover, pseudocopulation of bees and other hymenopterans which is induced by form and aphrodisiacal odor of orchid flowers is well-documented (KULLENBERG, 1956, 1973; PRIESNER, 1973; KERR *et al.*, 1962).

Males of *L. zephyrum* perceive individual odor differences of females; these odors are aphrodisiacs and perhaps other female odors (BARROWS, 1975 *c*; BARROWS, BELL and MICHENER, 1975).

Aphrodisiac Production vs. Age. Similar experiments with impregnated papers and dot pouncing showed that females secrete the aphrodisiac at least from day 1 to day 8; no older bees were tested.

Source of Aphrodisiac. Experiments were made to ascertain which of the three body sections (head, mesosoma, metasoma) of females of *L. zephyrum* carry aphrodisiac. Females, killed by freezing, were dismembered into the three sections; appendages remained on respective sections. Each section was placed for 20 to 24 hours on a piece of moist filter paper (5 cm^2) in a closed 22 ml vial at room temperatures. Control papers were prepared by moistening and placing them in vials without bee parts for the same time period at the same temperatures. Papers in sets of four (one impregnated with odor from each of the sections, one control) were presented, each for 1 minute, in random order on dot cards to flight groups of 30 males in medium-sized cages.

All three body portions bore aphrodisiac since male response toward dots in the presence of papers impregnated with head odor, mesosoma odor, and metasoma odor was significantly greater than that in the presence of control papers ($p = .009$, $p < .005$, $p < .005$, respectively, WT). Moreover, male response toward odors of the three parts varied significantly ($\chi^2 = 22.864$, $df = 10$, $.025 > p > .010$, Friedman's randomized block test). During the experiment males pounced upon dots 329 times; 8.2 percent of these pounces were made during presentations of controls; 24.6 percent, head odor; 45.6 percent, mesosoma odor; and 21.6 percent, metasoma odor. It is not yet known where the aphrodisiac originates; however, mesosomas, with attached appendages and thus the largest surface area to which odor could adhere, seem to be more attractive than other body portions. It is possible that this odor is produced in one part of the body, and then spread over the surface of the integument by grooming and other movements. On the other hand, the possibility that odor originates from the general body surface cannot be dismissed.

VISUAL CUES

Since tethered females commonly moved considerably (crawled on substrate, buzzed wings, moved appendages) while they were presented to males, it was thought that female movements might attract males. To test this hypothesis, dead and living females were presented to males. Tethered females of *L. zephyrum* and of *A. pura,* placed individually in vials, were killed by freezing and were thawed immediately before tests were made. Living and dead females, 16 each, were alternately presented, each for 1 minute, to flight groups of 20 conspecific males in medium-sized cages. Living females usually moved, from stirring very slightly to frantically struggling. Female movement may have attracted males of *A. pura* since they approached living females significantly more than dead ones ($p = .01$, WT), males of *L. zephyrum* did not; male response (contact with females per approach per minute) toward dead females was not significantly different than that made toward living females ($p > .05$, $n = 8$ in two tests, WT). Based on all contacts (long and short), making females immobile by freezing them to death does not decrease their attractiveness to males. Thus there is no evidence that movement by females is important in increasing male contact with females under these conditions. FREE (1971) also found no evidence that movement of *Bombus* queens was important in attracting males.

With respect to long contacts (grasps and attempted matings only), males of *A. pura,* but not those of *L. zephyrum,* contacted dead females significantly more than living ones ($p = .004$, $p > .05$, respectively, n's $= 8$, WT). In *A. pura* this might have occurred because living females were aggressive toward males and dead ones, of course, were not.

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Dr. G. C. EICKWORT of Cornell University and Dr. MICHENER identified bees. Voucher specimens have been deposited in the Snow Entomological Museum, University of Kansas.

Dr. Ursula JANDER kindly prepared a German summary and translations of table and figure captions. Ms Audrey Arnold translated the title.

Many field studies were made at the University of Kansas Natural History Reservation.

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