

**Bioassay evidence for a sex attractant pheromone in the large carpenter bee, *Xylocopa varipuncta* (Anthophoridae: Hymenoptera)**R. L. MINCKLEY<sup>1</sup>, S. L. BUCHMANN<sup>2</sup> AND W. T. WCISLO<sup>1</sup><sup>1</sup>*Department of Entomology, University of Kansas, Lawrence, Kansas 66045-2119, USA*<sup>2</sup>*USDA-ARS Carl Hayden Bee Research Lab., 2000 E. Allen Rd, Tucson, Arizona, USA and Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721, USA*

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(With 1 figure in the text)

A field bioassay was conducted to test whether compounds produced in the thoracic gland of male *X. varipuncta* are involved in long-range mate attraction. Filter paper impregnated with the two most abundant compounds of the male gland was placed in sites comparable to those used by territorial males during the time of day when mating activity occurs. Females visited these filter paper 'lures' as often as they visited unmanipulated males in the study area. Males with the chemical mixture added to their territories did not receive more matings than males at territories where we had not added the chemical mixture. These findings and other studies, summarized herein, support the hypothesis that the male gland produces a pheromone which functions as a long-range sex attractant.

**Contents**

	Page
Introduction . . . . .	285
Materials and methods . . . . .	286
Results . . . . .	287
Bioassay . . . . .	287
Mating behaviour . . . . .	287
Flight activity . . . . .	289
Discussion . . . . .	289
References . . . . .	290

**Introduction**

Males of the carpenter bee, *Xylocopa varipuncta* (Patton), have a large exocrine gland in the posterior dorsal region of the mesosoma. The chemical composition of the product of this gland consists of three terpenoids; all trans-geranylgeraniol, all trans-farnesal and 3,7,11- methyl 1-2 7,10-dodecatrienal together make up 97% of the blend and comprise 56.2%, 34.6% and 6.2% of the total mixture, respectively (Andersen *et al.*, 1988).

The gland's function was hypothesized to be the production of a female attractant pheromone (Andersen *et al.*, 1988) because it is not found in females and because males emit a strong 'floral scent' while at their mating territories (Marshall & Alcock, 1981, pers. obs.). The widely dispersed males are often found occupying territories alone, and females search out these males (Marshall &

Alcock, 1981). Male dispersion has made bioassay difficult because females rarely visit any given male territory (Alcock & Smith, 1987) and usually only one or two males can be watched accurately in one day. Our study was facilitated by the discovery (by J. Alcock in 1988) of an aggregation of male territories on a ridgetop near Tempe, Arizona. This study area enabled us to observe several males simultaneously in their territories (Alcock & Smith, 1987; Alcock & Johnson, 1990). Our objective was to test whether the volatile chemical blend found in the dorsal mesosomal gland of male *X. varipuncta* attracts female conspecifics from great distances.

Evidence from male *X. varipuncta* reproductive behaviour strongly suggests the involvement of a pheromone (Alcock & Johnson, 1990; Minckley & Buchmann, 1990). For example, males establish their territories near the tops of plants and shrubs. In addition to being positioned near the apex of the plant canopies, males are usually found using the taller plants in an area or those plants located near the top of a ridge or hill. The use of such locations should enhance the dispersal of a pheromone (Minckley & Buchmann, 1990). Pheromone dispersal has also been proposed as an explanation of why males shift the locations of their territories in response to changes in wind direction (Alcock & Johnson, 1990).

### Materials and methods

We obtained and used the 2 most abundant compounds found in the male gland, all trans-geranyl geraniol and all trans-farnesal. Both compounds were supplied by the Kuraray Co., Ltd. (Shin-Nihonobashi Building, 8-2, 3-Chome, Nihonobashi, Chou-Ku, Tokyo, 103, Japan) and the purity, as determined by capillary gas chromatography (GLC), was 96.9% and 94.6%, respectively. We mixed these chemicals in the relative proportions in which they are found in the male gland, 61.5% all trans-geranyl geraniol and 38.5% all trans-farnesal.

Most of the observations were made between 14 and 23 March 1989, on Pima Ridge in South Mountain Park, Maricopa County, Arizona. This study area has been described in detail by Alcock & Smith (1987) and is an east-west ridge 1.5 km long. The common perennial plants are indigenous to the Sonoran Desert and included littleleaf palo verde (*Cercidium microphyllum*), acacia (*Acacia greggii*), mesquite (*Prosopis velutina*), creosote bush (*Larrea tridentata*) and saguaro (*Cereus giganteus*).

We presented the synthetic mixture to females in 50–100  $\mu$ l doses applied every 30 min to 3-cm diameter discs of Whatman No. 1 filter paper (Whatman Co.) with an Eppendorf pipette. After 30 min the filter paper, although dry to the touch, still smelled strongly of the mixture. The synthetic blend was placed in 2 types of location: (1) in a plant that a male *X. varipuncta* was using at the time as a territory site or; (2) in a nearby plant that was identical (e.g. in canopy volume) but that was not being used by a male as a territory site. The mixture was placed in the same plant species that males of *X. varipuncta* were using as territory sites and in comparable areas of the ridge. Plants used were creosote bush, mesquite, wolfberry (*Lycium andersonii*) and littleleaf palo verde. The pheromone-impregnated filter paper was placed at various heights (0.25–2.0 m) above the ground and always in the upper half of the plant.

As a control, observations were made of territorial males where no synthetic mixture had been added. In this paper we refer to these as 'unmanipulated territories' or 'unmanipulated males'.

Additional observations were made on 31 March 1989 at Tohono Chul Park, Tucson, Pima Co., Arizona. The synthetic mixture was placed in a mesquite tree not being used by a male *X. varipuncta*. Data were recorded as described previously for the South Mountain study.

All observations were made during the time of day when males are normally active at their territories (15:30 to 18:30 h MST). We continuously observed individual males until they left the area (i.e. focal individual sampling; Altmann, 1974). We used the same 3 categories of female behaviours as did Alcock & Johnson (1990). These were as follows: (1) 'passbys'—a looping flight within 2 m of a male territory; (2) 'close approach'—a female stopped within 0.5 m of the male for more than 1 s, facing him while hovering; and (3)

actual mating. Females that flew through the observation area quickly and did not change their flight path towards males or pheromone mixture sites were not used for the data analysis. Comparable observations were made of sites provided with only the chemical mixture.

We did not attempt to determine at what rate females visited plants that did not contain a male or a chemical lure because females do not visit such locations. Females in transit typically fly in a more or less straight path 1–3 m above the top of the vegetation and can be easily distinguished from an individual that has stopped, is searching for a foraging site or visiting a male lek site.

Observations suggested that male bees returned repeatedly to a specific area of the plant. Flight paths were characterized by analyses of videotapes of the flight paths of 4 territorial males that were made with a hand-held (General Electric Model number 9-9808 SE) video camera. Flight activity was digitally transcribed from a horizontal screen showing the video film, by means of a digitizing bit-pad connected to a computer; as the bee moved, a cursor pen was used to follow the bee and to input the path in real time at 11 data points per second.

## Results

### *Bioassay*

In the 90.25 h of observations, 38 female visits were recorded at locations with males (with and without the synthetic mixture present) or to locations with the synthetic mixture alone. Fifteen of these female visits were passbys, 19 were close approaches and four were matings. We found that females visited the synthetic mixture (with or without males present) as often as they visited the unmanipulated males (Table I). The synthetic mixture alone attracted females more frequently than either males alone or males with the synthetic mixture, but those differences were not significant ( $\chi^2 = 3.01$ ,  $P < 0.05$ ,  $d.f. = 1$ ; Table I).

Each female observed approaching the mixture behaved similarly to others seen in unmanipulated male-female interactions. All approached the filter paper from downwind and hovered within 5 cm of the volatile lure for several seconds (10, 5, 5 and 2 s in the cases of the four individuals) before departing. On two of the three occasions when we observed females visit male territories enriched with synthetic pheromone, the female approached the filter paper before approaching the resident male. One such occurrence resulted in a mating.

### *Mating behaviour*

The single copulation witnessed in a territory with supplemental pheromone was observed from a distance of 1.5 m; several details of the mating are new and are now described. The male was

TABLE I

*Frequency per hour of behaviour observed ( $\times 10^{-1}$ ) for the three experimental designs. Numbers of observations are included in parentheses. All values are not significantly different in a Chi-squared test from males without synthetic pheromone added. NA = not applicable*

Experimental design	Total min observed	Passbys	Close approaches	Matings	Total female interactions
Synthetic pheromone without male	633	1.06 (1)	3.79 (4)	NA	4.74 (5)
Synthetic pheromone with male	1082	1.11 (2)	1.66 (3)	0.06 (1)	3.33 (6)
Males without supplemental pheromone	3699	1.95 (12)	1.95 (12)	0.49 (3)	4.38 (27)

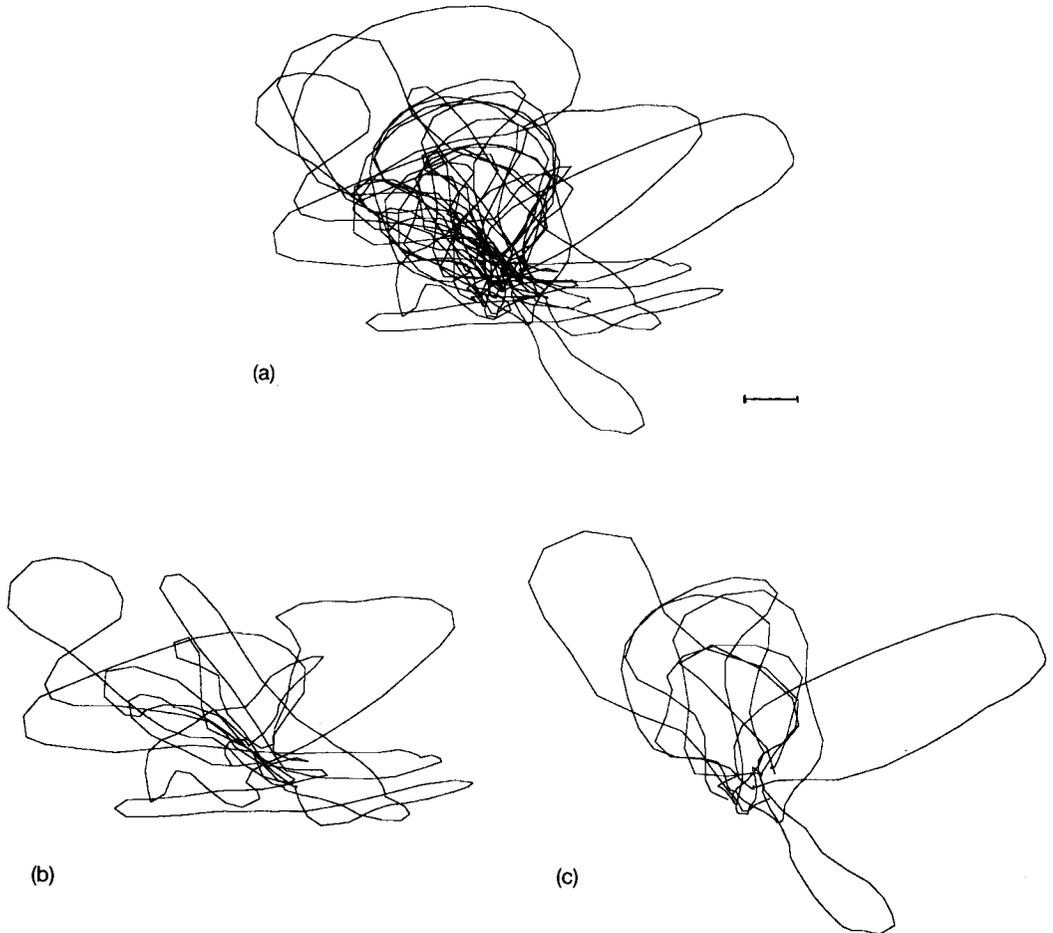


FIG. 1. Flight pathway of a territorial male *X. varipuncta* showing the repeat visits to a central area and the many directions of the short loop flights. The pathway is traced for 81 s in (a) and the same segment subdivided into lengths of 0–40 s in (b) and 41–78 s in (c). Scale bar = 14 cm.

hovering 1.5 m above ground level in a mesquite tree growing 8–10 m below the ridgetop in a rock-formed cul-de-sac. The female flew in from the top of the ridge (also from the downwind side), following the contour of a depression that extended from below the male's territory toward the ridgetop. This was a common pattern of female approach (Alcock & Johnson, 1990) and suggests that mate-location involved females following pheromonal plumes. The female first approached the filter paper 20 cm downwind from the male's position and hovered within 2–3 cm of it for > 5 s. She then landed on a twig 5 cm from the paper, and the male immediately landed on her dorsum, causing them both to drop to the ground. The copulation lasted 18 s and was accompanied by loud buzzes (presumably produced by the male's indirect flight muscles) at a rate of about one per second. Such an auditory component occurs in anthophorines and other bees (e.g. Alcock & Buchmann, 1985; Larsen, Gleffe & Tengo, 1986; Wcislo, Minckley & Spangler, In prep.) but has not been previously reported in *Xylocopa* except perhaps by Houston (1974) describing two

matings of a *Xylocopa* in the subgenus *Koptortosoma* (probably *X. aruana*); he says that the pairs were 'loudly buzzing' as they fell to the ground. The mating reported in our study was not preceded by the 'land and walk' male behaviour (Marshall & Alcock, 1981, pers. obs.). When visited by a female, males will often land on a plant, quickly walk upward for several centimetres, take flight and hover facing the female. This male behaviour may be repeated several times.

### *Flight activity*

A typical flight path of an undisturbed territorial male is shown in Fig. 1. In the middle of the figure is the focal area to which this male returned approximately every 3 s and where he often stopped briefly ( $\bar{x}=0.2$  s,  $n=7$  stops) before flying out in another loop. Activity of four males analysed from videotape for a total of 432 s had loop durations averaging 12.5 s ( $n=34$ ). Flights were punctuated by 0.35 s pauses (S.D. = 0.26,  $n=30$ ), usually in the central focal area. A central area that is visited after each loop is commonly observed at male territories, and is also where males hover upon the approach of females and usually land before copulation occurs (Alcock & Smith, 1987; this study).

### Discussion

The results of the bioassay are consistent with a hypothesis that the large male mesosomal gland of *X. varipuncta* produces volatile components which are attractive to female conspecifics. A synthetic chemical mixture of the two primary glandular components attracted female conspecifics as frequently as did unmanipulated males. Notably, females visited sites that had only the synthetic mixture and lacked territorial males as frequently as they visited unmanipulated males in their territories. These observations suggest that these compounds act as long-range primary olfactory attractants and male advertisements.

Whether female mate choice is based on an aspect of pheromone production or quality is unanswered by these data. We found that males with the supplemental synthetic mixture in their territories did *not* receive more matings than unmanipulated males; however, our small sample sizes preclude any statistical analysis.

The large mesosomal gland in this species is seasonally active, producing the three-component blend of terpenes a few days before the onset of male sexual maturity and the establishment of daily leks (S. L. Buchmann & R. L. Minckley, unpubl. obs.). Overwintering males and those collected within the first few days of flight activity in the spring have no detectable scent of terpene reserves within the gland lumen. In fact, within a few days the glands undergo rapid metabolic activity, changing from a non-secretory tissue the colour of adjacent flight muscles to highly active glands that empty their products into a multibranching lumen. This is followed by an apparent postsecretory phase in which the entire gland stops producing pheromone and becomes a darkened, sclerotized storage organ. Histological and SEM/TEM studies (S. L. Buchmann, D. Jimenez & R. L. Minckley, unpubl. data) are under way towards a further understanding of the rapid physiological changes that occur within these unique exocrine glands.

The pheromone may be one of several cues used by females searching for mates. Two visual cues that might also be involved are the locations of sites that males use for their territories and the golden colour of males of this species (in contrast to the black females). Tall trees and topographic high spots (prominent locations) may be where females search when they are reproductively active (Alcock & Smith, 1987). These observations of females flying to and hovering near (2–3, 5, 10 and 30 cm) filter paper impregnated with the synthetic pheromone mixture may indicate that close

visual inspection is not very important for locating mates. Alternatively, the light coloration could function primarily to decrease thermal stress during the energetically expensive hovering flight of male *X. varipuncta*, rather than as a cue for females.

Chemical marking of the plants in the territory is reported in some other species of *Xylocopa* (Gerling, Velthius & Hefetz, 1989; Vinson & Frankie, 1990) and there are some observations that suggest territorial male *X. varipuncta* mark the central area of their territories with chemicals. These observations are: (1) flying *X. varipuncta* males occasionally brush against leaves or twigs in the focal area; (2) the 'land and walk' behaviour occurs at the focal area and this behaviour may involve the application of a chemical; and (3) two separate observations were made in 1985 (by RLM) of single females flying to and pausing at an area of a non-flowering plant that had been the focal area of a male territory and abandoned during the preceding 10 min. Despite these observations, it is possible that marking, if it occurs, is unintentional because overt marking behaviour is not consistently observed when males first arrive at their territories as it is in *X. sulcatipes* (Hefetz, 1983), some bumblebees (Svensson, 1979) and wasps of the genus *Philanthus* (Evans & O'Neill, 1988). The source of the twig-marking chemicals is unknown and could be the thoracic or mandibular glands; the latter are enlarged in male *X. varipuncta*.

Our bioassay experiments cannot entirely discount the possibility that females perceived the synthetic chemical blend as some other attractant scent such as a floral odour. Even if this is so, the material could still function as a sex attractant; males may be taking advantage of female sensory apparatus that is already operational. Nevertheless, this bioassay experiment adds further evidence to that from male reproductive behaviour and the timing of exocrine product production in the gland, that these chemical compounds *are* used as a sex attractant by male *X. varipuncta* for long-distance mate attraction.

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