Insectes soc. 49 (2002) 354–356
0020-1812/02/040354-03

Insectes Sociaux

Research article

Chemical trails used for orientation in nest cavities by two vespine wasps, *Vespa crabro* and *Vespula vulgaris*

I. Steinmetz, S. Sieben and E. Schmolz*

Freie Universität Berlin, Institut für Zoologie, Königin-Luise-Straße 1–3, D-14195 Berlin, Germany, e-mail: eschmolz@zedat.fu-berlin.de

Received 2 August 2001; revised 3 December 2001 and 28 May 2002; accepted 14 June 2002.

Summary. Foragers of the two cavity-nesting vespines, *Vespa crabro* and *Vespula vulgaris*, lay and follow chemical trails in their nest cavities. In our experiments 75% of *V. crabro* and 72% of *V. vulgaris* foraging workers followed a trail arranged in a direction to which they were not accustomed. In experiments with trails taken in from conspecific alien colonies, 21% of *V. crabro* foragers and 65% of *V. vulgaris* foragers followed the trails. These behavioural responses indicate that the trail of *V. crabro* is colony-specific whereas that of *V. vulgaris* is not.

Key words: Vespidae, *Vespa crabro*, *Vespula vulgaris*, cavity nesting, trail-following pheromone, orientation.

Introduction

In Hymenoptera species with winged foragers knowledge about trail pheromones is rarely found. Several species use colony specific chemical cues for recognition of their nest entrances (Butler et al., 1969 for *Apis mellifera* and *Vespula vulgaris*; Foster and Gamboa, 1989 for *Bombus occidentalis*; Steinmann, 1976 for solitary bees). Evidence for the existence of chemical trails outside the nest are known from Meliponinae (Lindauer, 1956), Polistinae (Naumann, 1975; Jeanne, 1981, 1991; Kojima, 1994) and *Bombus* (Cederberg, 1977; Cameron et al., 1999). There are no data reported about trail production in the Vespinae. In contrast to vespine species constructing exposed nests, nest entrances of cavity breeding species, such as *Vespa crabro* Linnaeus and *Vespula vulgaris* Linnaeus, cannot be approached by flying since the nest entrance and the cavity entrance are spatially separated (Matsuura, 1991; Greene, 1991). Almost all the foragers walk the distance between nest and cavity entrance using the same path on the substrate, even where the space of the cavity is large enough to allow flying (pers. obs.). Furthermore, the light regime in nest cavities usually is very low and visual orientation for finding the nest is limited (pers. obs.). This leads to the question, how do foragers orient towards the nest.

In this study we examined whether *Vespa crabro* and *Vespula vulgaris* use terrestrial trail pheromones for orientation in nesting cavities or entrance tunnels and whether the trails are colony specific or not.

Methods

Collection of colonies

The experiments were carried out between July 1998 and October 2000 with three colonies of *Vespa crabro* and two colonies of *Vespula vulgaris* that were relocated from their natural sites (soil-, tree- or other cavities). The nests were transferred to wooden nest boxes (40 × 40 × 60 cm^3), which were placed in a laboratory located in the garden of the Institute for Zoology in Berlin-Dahlem.

To simulate light conditions typically found in nesting cavities, the laboratory was darkened to 3–15 lux (measured by luxmeter Dr. B. Lange, Berlin, type 4162). Colony size during testing ranged between 20 and 100 workers in the three *Vespa crabro* colonies and between 70 and 1300 foragers in both *Vespula vulgaris* colonies (number estimated according to Malham et al., 1991).

Evidence for a chemical trail used for orientation

Leaving for foraging flights and returning to their nest boxes, foragers of three *V. crabro* colonies and two *V. vulgaris* colonies had to pass through an experimental system (length about 1.5 m) consisting of transparent plastic tubes and three glass boxes (Fig. 1). Corresponding to their different body sizes, the tubes for *V. crabro* were 5 cm and for *V. vulgaris* 2.5 cm in diameter. By closing two of the three tubes only one possible path was offered to the workers for passing through the experimental system. By the second day after nest relocation most of the foragers crossed each glass box along a straight path on the movable glass plates at the bottom of the boxes. After five days in *V. crabro* and two days in *V. vulgaris*, a slight oily trail was visible at the area of the glass plates where the foragers had walked frequently.

* *Author for correspondence.*

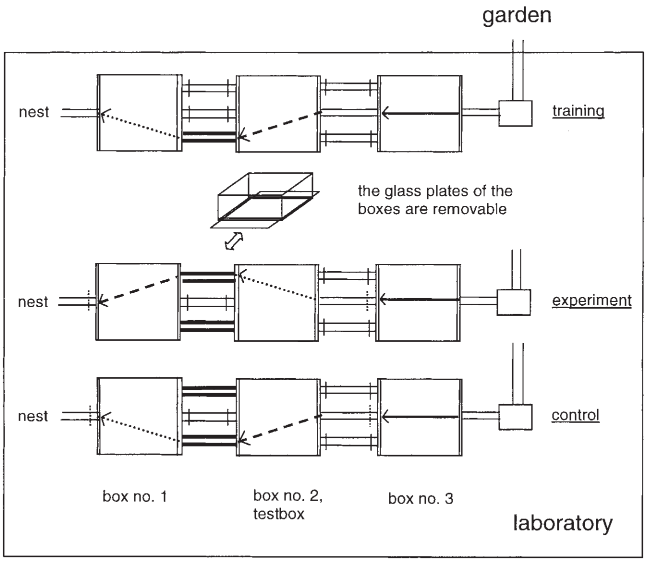

Figure 1. Scheme of the experimental setup, view from top. The pathway of homecoming *V. crabro* foragers and *V. vulgaris* foragers through the boxes is marked by arrows. The arrows correspond to the position of the oily trail. By exchanging the glass plates of box no. 1 and 2 the direction of the trail was varied. Opened tubes are marked by thick lines, tube lock mechanism is represented by vertical broken lines

The direction of this visible trail was changed from left to right for testing periods of five minutes by rotating (180°) and exchanging mutually the glass plates of boxes no. 1 and 2 (Fig. 1).

In three colonies of *V. crabro* and two colonies of *V. vulgaris* the behaviour of homecoming foragers was observed in box no. 2 (Fig. 1). In the experiments, every forager entering box 2 had to choose between (a) following the path along which they were trained (i.e. to the left toward the left-hand exit tube) and (b) following the path toward the right-hand exit tube. During the experimental tests (Fig. 1, middle), the marked chemical trail led to the right, i.e., in the direction opposite the training direction. During the controls (Fig. 1, bottom), the marked chemical trail led to the left, i.e., in the training direction.
Three behavioural categories were defined:

1) *walking to the right (= trail direction in the experiments):* passing at least 3/4 of the glass plate (this distance was marked on the bottom side of the glass plate in test box no. 2) towards the right-hand exit tube, no stopping, no flying
2) *walking to the left:* passing at least 3/4 of the glass plate in original direction towards the left-hand exit tube, no stopping, no flying
3) *unoriented:* running to the middle of the three tubes, flying, walking in an uneven manner or turning back before having passed 3/4 of the glass plate in one straight direction.

During each test period of five minutes with rotated glass plates, the number of foragers that followed each of these behavioural categories was recorded. Each test period was followed by a 5-minute period in which the glass plates were in their original position with the trail leading in the training direction (left) and both tubes left open (control period). During the control periods, the orientation behaviour of incoming foragers was also recorded according the above-mentioned categories. The assay was repeated 10 times in each colony. In order to avoid habituation to the test conditions, an interval of at least 15 minutes was taken between the successive assay experiment (including test and control periods) where the glass plates were in training position but only the left tube was left open.

Social influences on forager orientation during testing and control periods were ruled out by using microscope-slide gates to allow only one forager at a time to enter into the test box (Fig. 1).

Colony-specificity of the chemical trail

Two *V. crabro* – and two *V. vulgaris* – nests were used for experiments, each one placed simultaneously with its own system of boxes and tubes in the laboratory (for further description see above). Thus it was possible to put a glass plate used by the foragers of one colony into the test box of another colony and vice versa. The plate was placed in box no. 2 of the test colony, so that the trail (laid by the members of the foreign colony) now led in a novel direction (to the right tube). Incoming foragers of the two test colonies of *V. crabro* and *V. vulgaris* were tested in box no. 2 and their orientation behaviour was recorded for five minutes and assigned to the three categories defined above. In each colony, the assay was repeated 10 times.

Statistical analysis

Bioassay data were analysed by means of the Bonferroni corrected Chi-square test (Zar, 1984).

Results

Evidence for a chemical trail used for orientation

During the control situation more *V. vulgaris* foragers than *V. crabro* foragers were unoriented, but in both species the majority of the foragers crossed the test box straight in original trail direction. None of the foragers walked to the tube on the right (novel direction). The response rates of *V. crabro* and the *V. vulgaris* foragers on a switched trail were similar to each other and differed from their behaviour in the controls: the majority of the workers (about 75%) walked the novel direction to the right tube following the trail, only some crossed the test box in original direction to the left, and some workers were confused and unoriented (Table 1).

Colony-specificity of the chemical trail

The trail-following rate of workers on a trail laid by members of their own colony was similar in *V. vulgaris* and *V. crabro* (see above). Differences between *V. crabro* and *V. vulgaris* appeared in experiments with trails derived from alien conspecific colonies: Whereas the reaction of *V. vulgaris* foragers to a trail in novel direction (right) from an alien colony was not different from that to their behaviour on the own trail, in *V. crabro*, significantly fewer foragers followed the foreign trail than their own trail. Corresponding to this, the number of unoriented foragers on a foreign trail was higher in *V. crabro* than in *V. vulgaris* (Table 1).

Discussion

In the controls, the wasps chose the "correct" path, following the chemical trail or using topographic information that they had learnt during the "training", or both. The experiments with the switched trail showed that the wasps very likely use chemical trail for orientation in the cavity. Some of the *V. crabro* and *V. vulgaris* workers did not follow the novel trail

Table 1. Percentage of *V. crabro* foragers and *V. vulgaris* foragers crossing the test box towards the right-hand exit tube (trail direction in experiments), towards the left-hand exit tube (trail direction in the controls) or being unoriented. In brackets: number of tested colonies (n) and total number of foragers. For further details see text

		Vespula vulgaris				*Vespa crabro*		
		original trail direction to the left	unoriented	novel trail direction to the right		original trail direction	unoriented	novel trail direction
control	a	90% (n = 2; 353)	10% (n = 2; 39)	0% (n = 2; 0)	d	98% (n = 3; 298)	2% (n = 3 ; 6)	0% (n = 3; 0)
test with own trail	b	17% (n = 2; 45)	11% (n = 2; 29)	72% (n = 2; 193)	e	13% (n = 3; 35)	12% (n = 3; 32)	75% (n = 3; 200)
test with alien trail	c	19% (n = 2; 45)	16% (n = 2; 38)	65% (n = 2; 153)	f	22% (n = 2; 145)	57% (n = 2; 374)	21% (n = 2; 138)

Chi-square goodness of fit for 3 categories: a and b: $p < 0.001$; b and c: $p = 0.155$; c and f: $p < 0.001$. d and e: $p < 0.001$; e and f: $p < 0.001$. a and d: $p < 0.001$; b and e: $p = 0.406$

direction but crossed the testbox in the original direction. Despite the reduced light regime visual information and/or thigmotaxis also seemed to play a role in finding the route back to the nest. However, the trail is certainly an important parameter used by the majority of the workers for orientation. Our study delivers the first proof for trail following in the close vicinity of a nest in the Vespinae.

Our investigations showed that in *V. vulgaris* trail following rates on a trail of the home colony and on a trail laid by members of a foreign colony were not different. In *V. crabro*, however, only a small proportion of foragers oriented themselves by a trail of a foreign colony and the rate of unoriented foragers was higher compared to a trail of their own colony. We postulate that the trail of *V. vulgaris* does not transmit colony-specific information whereas the trail of *V. crabro* is a mixture of anonymous (eliciting trail following behaviour) and specific components (transmitting colony specific information) (after Hölldobler and Carlin (1987)). Colony specificity of the trail in *V. crabro* strongly indicates that the trail is not visual or tactile, but is perceived chemically by the foragers, thus consists of trail pheromones.

Acknowledgements

We thank Wolfgang Hoffmann and Lars Podsiadlowski for helping in relocating the nests, Ingolf Lamprecht, Joachim Ruther, Bob Jeanne and two anonymous referees for helpful comments on the manuscript. This study was supported by NaFöG, granted to I.S. and St.S.

References

Butler, C.G., D.J.C. Fletcher and D. Watler, 1969. Nest-entrance marking with pheromones by the honeybee, *Apis mellifera* L. and by a wasp, *Vespula vulgaris* L. *Anim. Behav. 17*: 142–147.

Cameron, S.A., J.B. Whitfield, M. Cohen and N. Thorp, 1999. Novel use of walking trails by the amazonian bumble bee, *Bombus transversalis* (Hymenoptera: Apidae). *Univ. Kansas Nat. Hist. Mus., Special Publ. 24*: 187–193.

Cederberg, B., 1977. Evidence for trail marking in *Bombus terrestris* workers (Hymenoptera, Apidae), *Zoon 5*: 143–156.

Foster, R.L. and G.J. Gamboa, 1989. Nest entrance marking with colony specific odors by the bumble bee *Bombus occidentalis* (Hymenoptera: Apidae). *Ethology 81*: 273–278.

Greene, A., 1991. *Dolichovespula* and *Vespula*. In: *The Social Biology of Wasps*, K.G. Ross and R.W. Matthews, (Eds.). Comstock Publishing Associates, Ithaca and London: pp. 263–305.

Hölldobler, B. and F. Carlin, 1987. Anonymity and specificity in the chemical communication signals of social insects. *J. Comp. Physiol. 161*: 567–581.

Jeanne, R.L., 1981. Chemical communication during swarm emigration in the social wasp *Polybia sericea* (Olivier). *Anim. Behav*. *29*: 102–113.

Jeanne, R.L., 1991. The swarm-founding Polistinae. In: *The Social Biology of Wasps*, K.G. Ross and R.W. Matthews, (Eds.). Comstock Publishing Associates, Ithaca and London: pp. 191–231.

Kojima, J.I., 1994. Behaviour during artificially induced swarm emigration in an Old World polistine wasp, *Ropalidia romandi* (Hymenoptera: Vespidae). *J. Ethol. 12*: 1–8.

Lindauer, M., 1956. Über die Verständigung bei Indischen Bienen. *Z. vergl. Physiol. 38*: 521–557.

Malham, J.P., J.S. Rees, P.A. Alspach, J.R. Beggs and H. Moller, 1991. Traffic rates as an index of colony size in *Vespula* wasps. *New Zealand J. Zool. 18*: 105–109.

Matsuura, M., 1991. *Vespa* and *Provespa*. In: *The Social Biology of Wasps*, K.G. Ross and R.W. Matthews, (Eds.). Comstock Publishing Associates, Ithaca and London: pp. 232–262.

Naumann, M.G., 1975. Swarming behaviour: evidence for communication in social wasps. *Science 189*: 642–644.

Sachs, L., 1997. *Angewandte Statistik*. Springer Verlag, Berlin, Heidelberg, New York.

Steinmann, E., 1976. Über die Nahorientierung solitärer Hymenopteren: Individuelle Markierung der Nesteingänge. *Mitt. Schweiz. Entomol. Ges. 46*: 253–258.

Zar J.H., 1984. *Biostatistical Analysis*, 2nd ed., Prentice-Hall, Englewood Cliffs, New Jersey.