

Research article

Changes in food source profitability affect Nasonov gland exposure in honeybee foragers *Apis mellifera* L.

P.C. Fernández and W.M. Farina*

Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, (C1428EHA) Buenos Aires, Argentina, e-mail: walter@bg.fcen.uba.ar

Received 26 July 2000; revised 12 March and 21 July 2001; accepted 1 August 2001.

Summary. When arriving at a known artificial food source, foraging honeybees usually perform circular flights around the feeding place prior to landing. During these flights bees expose their Nasonov gland, an exocrine gland located at the base of the 7th tergum, that releases a complex blend of volatiles. This behavior may continue even after the bee starts food ingestion. The proportion of bees exposing the Nasonov gland and the duration of its exposure before and during feeding for individual bees were quantified. Trained bees collected sugar solution during 12 visits from a feeder located at 160 m from the hive. Five different reward programs were presented: three constant and two variable. The constant programs offered 0.6, 1.2 or 2.4 M sugar for all 12 visits, while the variable programs delivered either 0.6, 1.2, 0.6 M or 0.6, 2.4, 0.6 M, four visits for each molarity. Results showed that sugar concentration changed the thresholds and durations of Nasonov gland exposure. However, this relationship was found only for Nasonov exposure before bees began to feed. During feeding, a protruded Nasonov gland was only observed for bees that had exposed it prior to feeding; suggesting that Nasonov gland exposure before feeding is a releaser of the during-feeding exposure. In variable reward programs, changes in sugar concentration were followed by changes in both thresholds and durations of exposure. However, Nasonov gland exposure during feeding did not appear to decrease based on measurements of the low profitability during the current foraging visit. These results suggest that Nasonov gland exposure is programmed on the basis of reward expectations, with the bees having acquired this information in the previous foraging visits to the food source.

Key words: *Apis mellifera*, honeybees, Nasonov gland, communication, foraging.

Introduction

Scent marking with pheromones plays a central role in the organization of insect societies (Wilson, 1971). In the context of foraging, there is some evidence suggesting that social insects leave chemical ‘signposts’ around a feeding place and that this scent marking behavior can be modulated by food source profitability. For example, ants of *Solenopsis geminata* (Fabricius) and *Acanthomyops interjectus* (Mayr) adjust the quantity of pheromone laid in their trails according to the quality of the reward. This modulation allows these ants to regulate the number of workers recruited to the food source (Hangartner, 1970).

Honeybees, *Apis mellifera*, produce numerous attractive compounds used for orientation during foraging such as footprints, sting-produced compound, forage marking pheromone and Nasonov gland compounds (Winston, 1987). In this study we focus exclusively on scent marking by the complex of volatile substances released by the Nasonov gland. This gland is located dorsally at the level of the 7th abdominal tergum (Snodgrass, 1956). It consists of a mass of large glandular cells, which secrete a mixture of geranic and nerolic acid, geraniol, nerol, farnesol and citral through 600 min ducts into a canal (Free, 1987). The Nasonov gland and canal are usually concealed by the overlapping part of the 6th tergum and the bee exposes them by flexing the tip of its abdomen downward (Free, 1987). Nasonov gland exposure occurs in a variety of circumstances related to the orientation of colony members, such as when bees are in a swarm that is moving or forming a cluster, at the hive entrance (Free, 1987), collecting water (Free and Williams, 1970), or foraging at food sources. However, few observations of bees exposing their Nasonov gland during nectar foraging have been documented from natural sources (Frisch, 1967). Under experimental conditions, Nasonov scenting is modulated by the concentration and flow rate of sugar solution offered at a food source (Pflumm, 1969; Núñez,

* Author for correspondence.

1971a). Moreover, the tendency to expose the gland greatly differs among individuals of the same colony: some bees never expose their Nasonov gland even after performing numerous consecutive foraging trips, while others consistently expose this gland after a new profitable source is found (Free, 1968). Additionally, factors such as the food intake rate of the hive (Pflumm, 1969; Pflumm et al., 1978), the presence of a floral (Free, 1968; Wenner and Wells, 1990), or the Nasonov gland scents (Free, 1968), and environmental conditions (Núñez, 1971a) may affect Nasonov gland exposure.

When collecting nectar honeybees may expose their Nasonov gland before and/or after probing the sugar solution at a feeding place (Frisch, 1967; Free, 1968; Pflumm, 1969; Núñez, 1971a). Pflumm (1969) described three different behavioral sequences for bees exposing the Nasonov gland for different profitability ranges: 1) when foraging at low-profit food sources, bees fly straight to the artificial feeder and land there without flying around it. When they land, the scent organ is not exposed. 2) For higher food source profitability at the feeding place, bees fly in circles around the food source while exposing their Nasonov gland before feeding. 3) If the food source profitability is even higher, the bees expose their Nasonov gland while flying and they keep the organ everted for part of the feeding time. Moreover, there may be two phases of Nasonov gland exposure after landing: in the first, the arrived bee fans its wings with the gland exposed before it begins to feed. In the second phase the bee exposes the Nasonov gland during part of the feeding, with or without fanning (Pflumm, 1969). Although some works studied Nasonov gland exposure during the flight and the feeding stages (Frisch, 1967; Free, 1968; Pflumm, 1969; Núñez, 1971a), none has identified a possible relationship between them. Free (1968) observed that bees that extensively exposed their glands before feeding also spent more time exposing them during nectar feeding. Therefore, our first goal was to quantitatively analyze both stages of the Nasonov gland exposure, before and during feeding, in bees confronted with different nectar rewards and determine if a relationship exists between them.

It is well known that the reward expectation that bees acquire throughout successive foraging visits may affect the estimation of food source profitability (Greggers and Menzel, 1993). For example, bees elicit different dance behaviors according to their previous foraging experience at the feeding site (Raveret Richter and Waddington, 1993). Thus, foragers may modify their Nasonov gland exposure according to not only the current food source profitability but also their previous experience. In order to examine this possibility, we analyzed whether abrupt fluctuations in food source profitability affected Nasonov gland exposure before and during feeding. These quantitative changes in profitability would allow us to understand how coupled both behavioral stages are.

Materials and methods

Experiments were performed at the experimental field of the University of Buenos Aires (34°32'S, 58°26'W) from March to April 1997 when competition with natural nectar sources is reduced. A colony of nearly 2000 European bees (hybrid descendents of *Apis mellifera ligustica* bees) was located in a single-frame observation hive (see Frisch, 1967).

Apparatus

The experimental food source was located 160 m from the hive in an open and shady place, conditions that allowed us to observe clearly the scenting flights of the experimental bees. It consisted of an artificial feeder delivering sucrose solution scented with almond essential oil (80 µl/L). Scented solutions were used both during training and assay. They were delivered at a constant rate of 5 µl/min through the center of a 40-mm-diameter disk of yellow paper. A synchomotor activated a pumping system, with a syringe delivering the sugar solution through a cannula (Núñez, 1971b). The syringe containing the sugar solution could be replaced when the bee was in the hive. In this way, the same 'artificial flower' was able to offer different sugar solutions (0.6, 1.2 or 2.4 M). Because these were provided at the same flow rate (5 µl/min), different amounts of sugar were delivered per unit time (1, 2 and 4 mg/min). During the assays, the feeder was switched on when the bee arrived at the feeding area, and off when it left for the hive. Therefore, sugar solution did not accumulate between foraging visits.

Procedure

A group of bees was trained to collect sugar solution from the artificial feeder and marked with a colored tag on the thorax. During each experimental day only one bee was selected for the measurements, while the others were caged for later use. The selected bee was allowed to freely visit the feeder and was recorded during 12 successive visits. The measurements took place between 1030 and 1330 h.

At the end of an assay, the caged bees were released at the feeding place, offering the same solution flow rate and sugar concentration than the following experimental day. Out of the experimental time, the apparatus was controlled automatically, switching on at 700 and off at 1800 h. Thirty minutes before the beginning of the experimental period (i.e., three complete foraging cycles), only a single, marked test bee was allowed to collect solution at the feeder under the same conditions as during the training period. This procedure eliminated perturbations caused by other bees, which may have influenced the performance of the test bees.

Experimental series

Three constant and two variable reward programs were alternatively offered during the two months of the experimental period. All of them delivered sugar solution at a rate of 5 µl/min during 12 successive foraging visits. For constant-reward programs the concentration of the solution was 0.6, 1.2 or 2.4 M. For variable-reward programs the concentration was changed after every four visits by the test bee. One program was 0.6–1.2–0.6 M and the other was 0.6–2.4–0.6 M.

Behavioral responses

Based on the behavioral sequences described by Pflumm (1969) the following behavioral parameters were recorded:

1) *Duration of Nasonov gland exposure before feeding*: the time the individual bees flew in circles close to the feeder. When bees fly around the food source it is only occasionally possible to clearly observe the gland everted, nevertheless, the scenting flights are very distinctive (see

Frisch, 1967; Free, 1968; Pflumm, 1969; Núñez, 1971a). Thus the time of circling flight around the feeder was used as an indirect measure of the duration of Nasonov gland exposure before feeding. Because it is impossible to accurately measure the precise time a bee begins to expose its Nasonov gland while flying, timing begins when a bee first approached to within 1 m of the feeder (Pflumm, 1969, Núñez, 1971 a). This measurement ended when bees started the ingestion of sugar solution at the feeder.

2) *Duration of Nasonov gland exposure during feeding*: the time individual bees exposed their Nasonov gland after having started the ingestion of sugar solution at the feeder (Pflumm, 1969; Núñez, 1971 a). Wing fanning was seldom observed in the present study, however, cases in which it was observed were included according to the categories defined above.

3) *Proportion of bees scenting*: This was defined as the proportion of bees that exposed their Nasonov gland before feeding (Free and Williams, 1972). This proportion was obtained by dividing the number of bees that exposed the gland by the total number of bees arriving at the source. This proportion was obtained for each of the twelve feeding visits recorded.

Statistical analysis

In the constant reward programs, sample sizes were 6, 6 and 5 bees at concentrations of 0.6, 1.2 and 2.4 M respectively. In the variable reward programs sample sizes were 6 and 9 bees for 0.6–1.2–0.6 M and 0.6–2.4–0.6 M respectively. To compare proportions of scenting bees over time we used Cochran's Q-test (Sokal and Rohlf, 1981). For each constant program, the proportion of scenting bees of the 12 visits was pooled, the three programs were compared by heterogeneity G-test (Sokal and Rohlf, 1981). For comparisons between duration of Nasonov gland exposure before and during feeding we used two-way repeated-measures ANOVA (Winer, 1971). Because the test of sphericity was rejected, we adopted the Huynh-Feldt adjusted probabilities for the within-subjects effects. Planned comparisons (orthogonal contrasts) were performed between the visit in which the sugar concentration was changed and the successive ones (i.e., the 5th and 6th–8th visits or the 9th and 10th–12th visits). In order to analyze the duration of scent marking as a function of sugar concentration, data of the 12 visits per bee in constant programs were pooled and analyzed by one-way ANOVA. In all cases, the ANOVA and planned comparisons were analyzed by the STATISTICA program (StatSoft Inc., 1999).

Results

Within each of the three constant reward programs (Fig. 1A) there was no change in the proportion of bees scenting with time (0.6 M: $Q = 17.9$, $df = 11$, NS; 1.2 M: $Q = 10.08$, $df = 11$, NS; and for 2.4 M: $Q = 9.26$, $df = 11$, NS). However, there were significant differences between the three programs ($G_H = 35.03$, $df = 2$, $p < 0.05$). Moreover, during the course of each of the variable reward programs (Fig. 1B–C), changes in the sugar concentration were followed by changes in the proportion of bees scenting (0.6–1.2–0.6 M: $Q = 28.03$, $df = 11$, $p < 0.005$; 0.6–2.4–0.6 M: $Q = 26.68$, $df = 11$, $p < 0.005$).

The duration of exposure before feeding for constant programs (Figs. 2A–C) differed between sugar concentrations ($F = 4.47$, $df = 2$, 10 , $p < 0.05$) but did not differ in the course of visits for any one sugar concentration ($F = 0.55$, $df = 11$, 110 , $p = 0.65$, NS) or the interaction between time and sugar concentration ($F = 0.47$, $df = 22$, 110 , $p = 0.83$, NS). By planned comparisons we found statistical differences between sugar concentrations (0.6 vs. 2.4 M: $F = 8.31$,

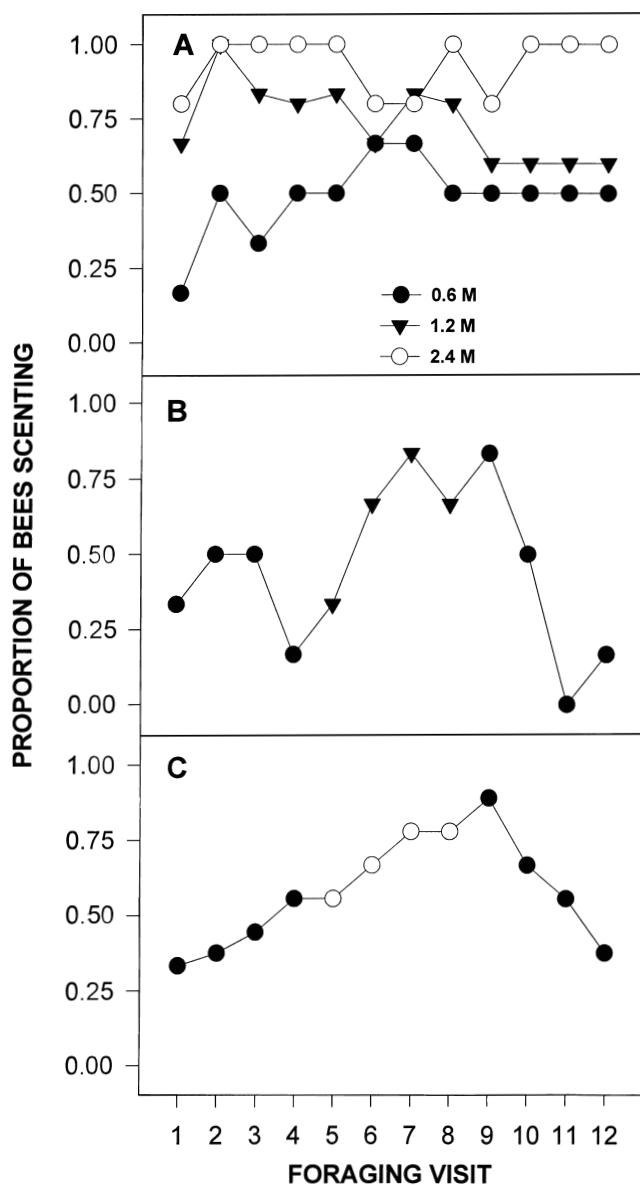


Figure 1. Proportion of bees scenting before feeding as a function of the successive foraging visits performed by individual trained bees. A) Constant reward programs offering 0.6, 1.2 or 2.4 M. B) and C) Variable reward programs offering 0.6, 1.2, 0.6 M or 0.6, 2.4, 0.6 M every four visits respectively. Black circles (●) indicate 0.6 M, black triangles (▼) indicate 1.2 M and white circles (○) indicate 2.4 M

$df = 1$, 10 , $p < 0.02$). The duration of exposure during feeding did not differ in either factor (sugar concentration: $F = 3.44$, $df = 2$, 10 , $p = 0.07$, NS; time: $F = 1.22$, $df = 11$, 110 , $p = 0.31$, NS) nor the interaction between them ($F = 1.49$, $df = 22$, 121 , $p = 0.22$, NS). In general, before-feeding exposure showed a more regular pattern along successive feeding visits than Nasonov gland exposure during it. Moreover, we observed exposure of Nasonov gland for the lowest sugar concentration (0.6 M) only before feeding, suggesting that the threshold to expose this gland is higher during feeding than before feeding.

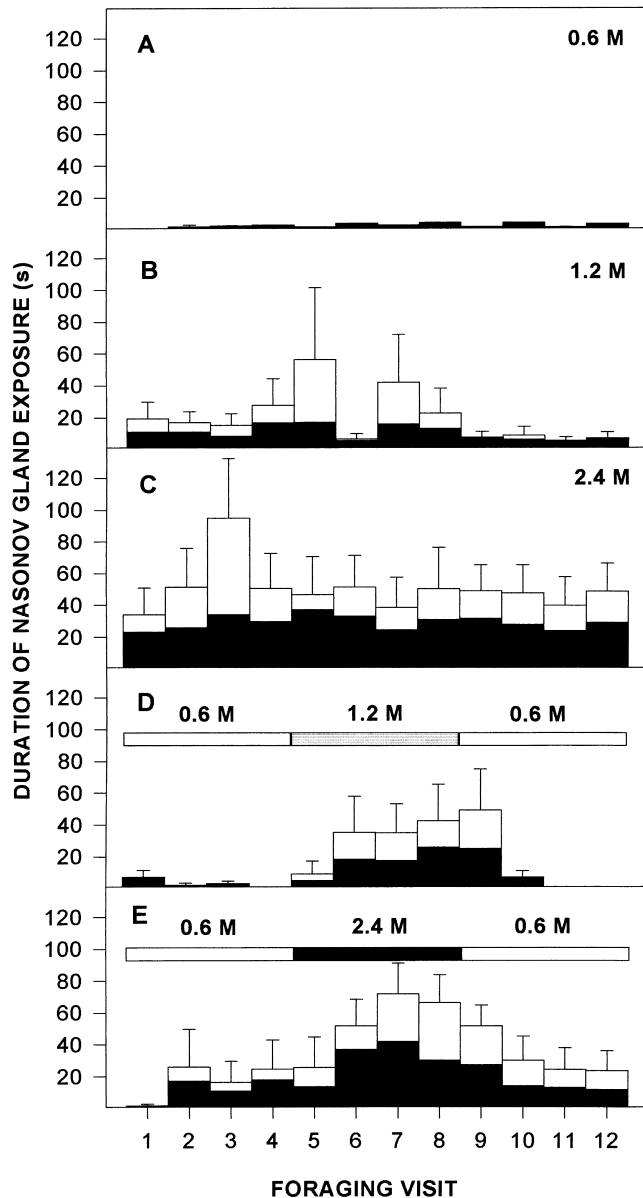


Figure 2. Duration of Nasonov gland exposure at the food source as a function of the different reward programs. The total duration in seconds, (mean \pm SE), is indicated by the sum of both bars: black, duration of the exposure before feeding and white, duration of the exposure during feeding. Constant reward programs: A) 0.6 M, B) 1.2 M and C) 2.4 M. In variable reward programs D) 0.6–1.2–0.6 M and E) 0.6–2.4–0.6 M, horizontal bars indicate the profitability offered in each visit: white, 0.6 M; gray, 1.2 M and black, 2.4 M

For the variable program of 0.6–1.2–0.6 M (Fig. 2D), we observed a significant increase in the duration of Nasonov-gland exposure before and during feeding among foraging visits (before feeding: $F = 2.71$, $df = 11, 55$, $p < 0.01$; during feeding: $F = 2.22$; $df = 11, 55$, $p < 0.05$) when sugar concentration increased. For the variable program 0.6–2.4–0.6 M (Fig. 2E), the duration of Nasonov gland exposure significantly differed among successive feeding visits both before and during feeding (before feeding: $F = 2.48$, $df = 11, 44$,

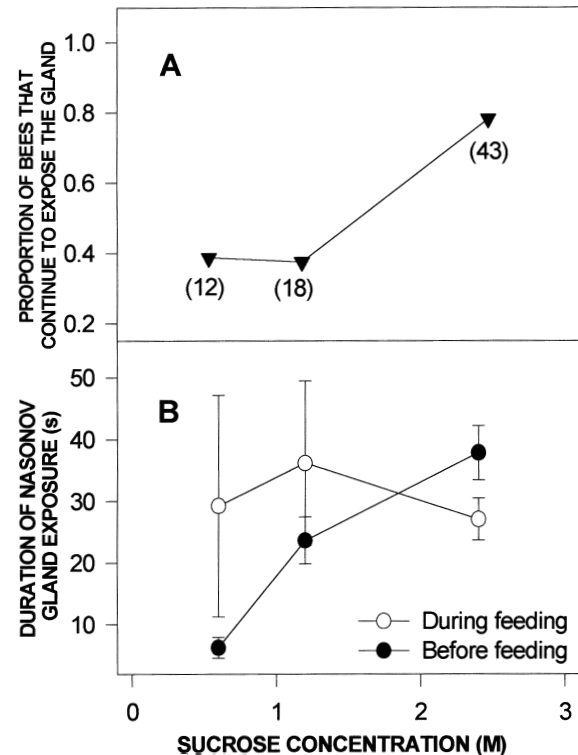


Figure 3. A) Proportion of bees that continue to expose the gland during feeding as a function of the sugar concentration offered. Data were obtained only from constant reward programs dividing the number of visits in which trained bees exposed their Nasonov glands before and during feeding by the number of visits in which they exposed their Nasonov glands only before feeding. Number of total visits in which bees exposed their Nasonov gland in parentheses. B) Duration of Nasonov gland exposure (s) as a function of the sugar concentration before (\circ) and during feeding (\bullet)

$p < 0.02$; during feeding: $F = 2.21$, $df = 11, 55$, $p < 0.05$). For before feeding, planned comparisons showed significant differences between the 9th and 10th–12th visits ($F = 10.43$, $df = 1, 4$, $p < 0.05$). For during feeding, significant differences were found between the 5th and 6th–8th visits ($F = 9.01$, $df = 1, 5$, $p < 0.05$). These results indicate that the duration of Nasonov gland exposure during feeding is not immediately adjusted according to the food source profitability offered. Moreover it was observed that during ingestion, Nasonov gland exposure only occurred in bees that had previously flown with the gland exposed. It is noteworthy that bees never exposed their glands during feeding (Fig. 2, white bars) without previously exposing it during flight (Fig. 2, black bars).

In order to study a possible temporal relationship between both kinds of Nasonov gland exposures, we analyzed for each sugar concentration, the proportion of bees that had previously exposed the gland before feeding and continued exposing the gland during feeding (Fig. 3A). This proportion depended on the profitability offered at the food source (i.e. it increased with the concentration, $G_H = 11.46$, $df = 2$, $p < 0.05$). The time of Nasonov gland exposure before feed-

ing depended on the concentration offered at the source (Fig. 3 B; $F = 6.56$, $df = 2, 66$, $p < 0.005$). However, the time spent exposing the gland during feeding was not affected by profitability and was very variable (Fig. 3 B; $F = 0.37$, $df = 2, 66$, $p = 0.68$).

Discussion

Our results show that sudden changes in profitability of food sources caused rapid changes in thresholds for Nasonov gland exposure by foraging honeybees. Moreover, once bees everted their glands, the duration of exposure also varied with profitability in a similar manner. However, this relationship was only found for Nasonov gland exposure occurring before bees began to drink at the feeder. During ingestion, Nasonov gland exposure was only observed in bees that had previously flown exposing it. No relationship was found between the time of exposure and the profitability of the rate-feeder for bees that continued exposing the gland while feeding.

The observed modulation of thresholds and duration of Nasonov gland exposure before feeding is comparable to the recruitment dance, in which thresholds and intensity of dancing may be modulated by changes in food source profitability (Waddington and Kirchner, 1992; Farina, 1996). These results support the idea of the Nasonov-gland exposure at the food source as a potential recruitment mechanism that can be modulated, in addition to dance, during foraging. In other words, Nasonov gland exposure before feeding may transfer quantitative information about the profitability of the exploited food source.

Considering that foraging bees are able to learn the characteristics of the exploited food sources, such as food source profitability, they may arrive at the feeding area with a certain expectation of the feeder's energetic reward. In our study, changes in food source profitability were accompanied by changes in Nasonov gland exposure with the time spent exposing the gland being re-adjusted during the next feeding visit. This is not surprising for the before feeding Nasonov gland exposure, since it is the first possible moment in which the forager can modulate its signaling behavior. In other words, when the bee leaves the hive towards the food source, its behavior seems to be programmed on the basis of reward expectations that are based on previous experience (i.e. the previous foraging visit). Thus, bees would arrive at the food source with a before feeding Nasonov-gland exposure program. These results are similar to other behaviors involved in the foraging cycle, such as crop-filling, dance maneuvers and trophallactic food exchange contacts that can be rapidly readjusted according to the current profitability offered by the source (Núñez, 1966, 1970, 1980; Raveret Richter and Waddington, 1993; De Marco and Farina, 2001). However, for the during-feeding Nasonov gland exposure, the time spent exposing the gland was not readjusted instantaneously even though bees could immediately perceive changes in profitability when they imbibed the sugar solution. During-feeding exposure was only readjusted during

the same visit than before-feeding exposure; suggesting a close relationship between these two behaviors. Moreover, Nasonov gland exposure during feeding was only observed for bees that had previously exposed the gland, an issue that suggests that Nasonov gland exposure before feeding is a necessary releaser of the during-feeding exposure.

Additionally, the during-feeding Nasonov gland exposure was highly variable, a fact that suggests that this stage of the scent marking may be affected by other factors not directly related to the food source profitability currently exploited. This hypothesis is supported by the result obtained by Pflumm and co-workers (1978) who found that Nasonov-gland exposure during feeding is highly affected by the nectar intake rate of the colony, a variable that was not controlled during the experiment.

From an informational point of view, the efficiency of Nasonov-gland exposure during flight around the feeding place as a communication channel may be higher than during feeding because the dispersion of volatile components is higher (Núñez, 1971 a). Although it has been suggested that Nasonov-gland exposure could actively enhance recruitment behavior at the food source (Frisch, 1923), no evidence has documented its capacity to recruit new comers to a feeding place per se. By extending this issue, our results have shown that the frequency and duration of exposure of the gland at a food source encode information about the profitability of that source. It remains to be seen whether bees in the colony use that information to increase foraging rate at that source.

Acknowledgements

We are deeply indebted to J.A. Núñez for his constant encouragement and fruitful comments and discussions throughout the study, and to M. Giurfa for many suggestions and corrections on early versions of the manuscript. We thank also G. Cogorno for invaluable help with the acquisition of data, A. Wainelboim for language help, Héctor Verna for providing technical support, and two anonymous referees for their valuable comments. This study was partially supported by funds from ANPCYT (PICT 98-03103) to WMF and by CONICET to both authors.

References

- De Marco, R.J and W.M. Farina, 2001. Changes in food source profitability affect the trophallactic behavior of forager honeybees (*Apis mellifera* L.). *Behav. Ecol. Sociobiol.* 50: 441–449.
- Farina, W.M., 1996. Food-exchange by foragers in the hive – a means of communication among honeybees? *Behav. Ecol. Sociobiol.* 38: 59–64.
- Free, J.B., 1968. The conditions under which foraging honeybees expose their Nasonov gland. *J. Apicult. Res.* 7: 139–145.
- Free, J.B., 1987. *Pheromones of Social Bees*. Chapman and Hall, London. 218 pp.
- Free, J.B. and I.H. Williams, 1970. Exposure of the Nasonov gland by honeybees (*Apis mellifera*) collecting water. *Behaviour* 37: 286–290.
- Free, J.B. and I.H. Williams, 1972. The role of the Nasonov gland pheromone in crop communication by honeybees (*Apis mellifera* L.). *Behaviour* 41: 314–318.
- Frisch, K. von, 1923. Über die „Sprache“ der Bienen, eine tierpsychologische Untersuchung. *Zool. Jb. (Physiol.)* 40: 1–186.

- Frisch, K. von, 1967. *The Dance Language and Orientation of Bees*. Harvard University Press, Cambridge, Mass. 566 pp.
- Greggers U. and R. Menzel, 1993. Memory dynamics and foraging strategies of honeybees. *Behav. Ecol. Sociobiol.* 32: 17–29.
- Hangartner, W., 1970. Control of pheromone quantity odor trails of the ant *Acanthomyops interjectus* (Mayr). *Experientia* 26: 664–665.
- McFarland, D.J., 1971. *Feedback Mechanisms in Animal Behaviour*. Academic Press, London. 279 pp.
- Núñez, J.A., 1966. Quantitative Beziehungen zwischen den Eigenschaften von Futterquellen und den Verhalten von Sammelbienen. *Z. verg. Physiol.* 53: 142–164.
- Núñez, J.A., 1970. The relationship between sugar flow and foraging and recruiting behaviour of honey bees (*Apis mellifera* L.). *Anim. Behav.* 18: 527–538.
- Núñez, J.A., 1971a. Beobachtungen an sozialbezogenen Verhaltensweisen von Sammelbienen. *Z. Tierpsychol.* 28: 1–18.
- Núñez, J.A., 1971b. Simulador para estudios del aprendizaje en la abeja *Apis mellifera* L. *Acta Cient. Ven.* 22: 101–106.
- Núñez, J.A., 1980. Comparación del comportamiento recolector de abejas africanizadas y abejas europeas. In: *Social Insects in the Tropics*. Proc. 1st Int. Symp. (P. Jaisson, Ed.) Cocoyoc, Morelos, Mexico. Vol 1, pp. 221–231.
- Pflumm, W., 1969. Beziehungen zwischen Putzverhalten und Sammelbereitschaft bei der Honigbiene. *Z. vergl. Physiol.* 64: 1–36.
- Pflumm, W., C. Peschke, K. Wilhelm and H. Cruse, 1978. Einfluss der – in einem Flugraum kontrollierten – Trachtverhältnisse auf das Duftmarkieren und die Abflugmagenfüllung der Sammelbiene. *Apidologie* 9: 349–362.
- Raveret Richter, M. and K.D. Waddington, 1993. Past foraging experience influences honeybee dance behavior. *Anim. Behav.* 46: 123–128.
- Snodgrass, R., 1956. *Anatomy of the Honey Bee*. Cornell University Press, Cornell. 334 pp.
- StatSoft, Inc., 1999. *STATISTICA for Windows* [Computer program manual]. StatSoft, Inc., Tulsa, OK, USA.
- Sokal, R.R. and F.J. Rohlf, 1981. *Biometry*, 2nd edn. Freeman, San Francisco. 859 pp.
- Waddington K.D. and W.H. Kirchner, 1992. Acoustical and behavioral correlates of profitability of food source in honey bee round dances. *Ethology* 92: 1–6.
- Wenner, A.M. and P.H. Wells, 1990. *Anatomy of a Controversy. The Question of Language among Bees*. Columbia University Press, New York. 399 pp.
- Wilson, E.O., 1971. *The Insect Societies*. Belknap Press of Harvard University Press, Cambridge, Mass. 548 pp.
- Winer, B.J., 1971. *Statistical Principles in Experimental Design*, 2nd edn. McGraw Hill.
- Winston, M., 1987. *The Biology of the Honey Bee*. Harvard University Press. Cambridge, Mass. 281 pp.



To access this journal online:
<http://www.birkhauser.ch>
