

# Interval Timing by an Invertebrate, the Bumble Bee *Bombus impatiens*

Michael J. Boisvert<sup>1,\*</sup> and David F. Sherry<sup>1</sup>

<sup>1</sup> Behavioral and Cognitive Neuroscience Group  
Department of Psychology  
University of Western Ontario  
London, Ontario N6A 5C2  
Canada

## Summary

Sensitivity to temporal information and the ability to adjust behavior to the temporal structure of the environment should be phylogenetically widespread. Some timing abilities, such as sensitivity to circadian cycles, appear in a wide range of invertebrate and vertebrate taxa [1, 2]. Interval timing—sensitivity to the duration of time intervals—has, however, only been shown to occur in vertebrates [3, 4]. Insect pollinators make a variety of decisions that would appear to require the ability to estimate elapsed durations. We exposed bumble bees to conditions in which proboscis extension was reinforced after a fixed duration had elapsed or after either of two fixed durations had elapsed. Two groups of bees were trained with a short duration (either 6 s or 12 s) and a long duration (36 s) in separate experimental phases (independent timing groups), whereas two other groups were trained with a short duration (either 6 s or 12 s) and long duration (36 s) always intermixed unpredictably (multiple timing groups). On long intervals, independent timing groups waited longer than mixed timing groups to generate the first response and responded maximally near the end of the interval. Multiple timing groups waited the same amount of time on average before generating the first response on both long and short intervals. On individual trials, multiple timing groups appeared to time either the long duration only or both the short and long durations: most trials were characterized by a single burst of responding that began between the short and long duration values or by two bursts of responding with the first burst bracketing the short value and the second burst beginning in anticipation of the long value. These results show that bumble bees learn to time interval durations and can flexibly time multiple durations simultaneously. The results indicate no phylogenetic divide between vertebrates and invertebrates in interval timing ability.

## Results and Discussion

All environmental events can be defined by their location in time, their position in a sequence, and their duration. Behavior is constrained by the temporal properties of the environment across a range of time scales, from seconds and minutes to days and years. Sensitivity to time

and the ability to adjust behavior to the temporal structure of the environment should therefore be phylogenetically widespread. Time, however, is also an abstract stimulus dimension processed by multiple sensory modalities [5, 6]. Responding appropriately to the passage of time may require the cognitive ability to analyze the temporal properties of experience. Sensitivity to elapsed intervals of seconds or minutes in duration has only been shown to occur in vertebrates [3, 4]. However, ecological considerations suggest that pollinating insects might possess keen interval timing abilities. Many pollinators show resource fidelity—the repeated use of renewing nectar sources [7–10]—and are therefore faced with the problem of scheduling revisits to resources with rewards that vary according to temporal schedules. Avian pollinators are indeed sensitive to temporal intervals of nectar replenishment [11, 12]. Here, we investigated whether an insect pollinator would show the ability to time intervals between successive food rewards and whether two durations could be timed concurrently.

The classic method for investigating interval timing by vertebrates is the fixed-interval (FI) procedure. In an FI procedure, an animal receives a reward on the first response that occurs after a fixed interval of time has elapsed since some time marker. In the FI procedure, a cue such as a light signals the start of a single interval to be timed. The cue is terminated, and a reinforcer is delivered on the first response that occurs after the programmed interval has elapsed. Two or more fixed intervals can be incorporated within a session to analyze the timing of multiple intervals concurrently. Vertebrates trained on FI procedures withhold responses for one-third to two-thirds of the interval duration, and when several intervals are aggregated, the maximum rate or probability of responding occurs at the end of the interval [13]. In a preliminary experiment with bumble bees in our laboratory, proboscis extension was reinforced after a single duration, either FI 12 s or FI 24 s. Bees' timing behavior was in many ways consistent with that of vertebrates trained on FI schedules. Mean wait time—the time elapsed until the first response—was longer on the FI 24 s duration than on the FI 12 s duration ( $F[1,4] = 31.91$ ,  $p < 0.006$ ), and in both cases, wait times occupied one-third of the interval durations. Wait times on the FI 24 s schedule were longer when bees had prior experience on the short schedule ( $F[1,4] = 16.20$ ,  $p < 0.017$ ). Maximal rate of responding occurred at or near the end of the interval on both schedules.

In more complex multiple timing procedures, vertebrate responding is often clustered around both the short and the long interval values [14–16], suggesting the ability to time both durations. Here, we extended our preliminary findings with bumble bees to investigate the timing of multiple concurrent durations. Bumble bees were trained on FI schedules by using an automated chamber connected to a bumble bee colony (Figure 1). A bee could collect a sucrose reward by extending its proboscis through a small hole in one wall of the

\*Correspondence: mboisver@uwo.ca

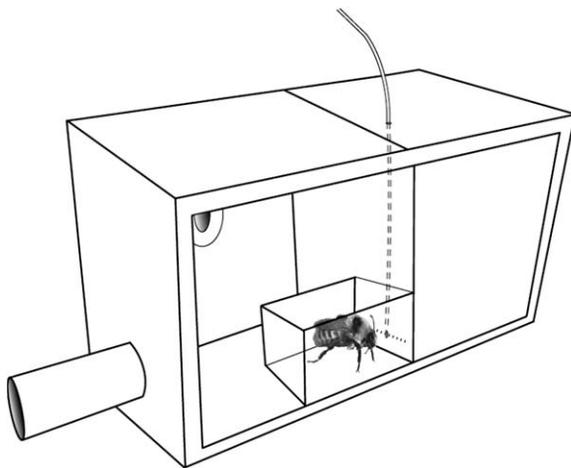


Figure 1. Schematic Diagram of Apparatus

A bee entered the chamber through a tube connected to the colony and was confined to a small space near the nectar source. Each extension of the proboscis through a hole in the wall of the chamber interrupted an infrared photobeam (dotted line) and was recorded as a response by the computer. A syringe pump delivered nectar through a fine tube (double dashed line). The first response that occurred after a fixed time interval had elapsed extinguished the light on the rear wall of the chamber and delivered a drop of sucrose to the tip of the syringe tubing. The apparatus measured 15 (total length)  $\times$  7.5 (width)  $\times$  8 cm (height).

chamber. After consumption of the initial reward, the chamber was illuminated by a light and a programmed interval began. The first proboscis extension that occurred after the interval elapsed caused the light to be extinguished and a sucrose reward to be delivered. Each bee completed several trials of this kind during a single foraging bout before returning to the colony. Four groups of bumble bees were trained on combinations of a short FI (6 s or 12 s) and a long FI (36 s). Two groups of bees experienced  $FI_{short}$  and  $FI_{long}$  intermixed in every session of the experiment. Two other groups experienced  $FI_{short}$  and  $FI_{long}$  sessions in separate phases of the experiment,  $FI_{short}$  in an initial phase, followed by  $FI_{long}$  in a subsequent phase. We analyzed wait times, defined as the delay to the first response during an interval, and rates of responding as a function of time since the start of the interval.

Wait times for independent and multiple timing conditions are shown in Figure 2. For the FI 6 s/FI 36 s combination, there were significant effects of FI (short versus long),  $F(1,6) = 87.74$ ,  $p < 0.001$ ; condition (independent versus multiple),  $F(1,6) = 27.32$ ,  $p = 0.002$ ; and FI  $\times$  condition,  $F(1,6) = 117.77$ ,  $p < 0.001$ . Post hoc tests showed that wait times were longer on  $FI_{long}$  than on  $FI_{short}$  for bees in the independent timing condition,  $t(3) = 10.14$ ,  $p < 0.005$ , but did not differ for bees in the multiple timing condition,  $t(3) = 0.97$ . The same pattern emerged for the FI 12 s/FI 36 s combination. There were significant effects of FI,  $F(1,6) = 12.50$ ,  $p = 0.012$ ; condition,  $F(1,6) = 17.36$ ,  $p = 0.006$ ; and FI  $\times$  condition,  $F(1,6) = 17.10$ ,  $p = 0.006$ . Wait times of bumble bees in the independent timing condition were longer on  $FI_{long}$  than on  $FI_{short}$ ,  $t(3) = 3.51$ ,  $p < 0.01$ , but did not differ in the multiple timing condition  $t(3) = 1.57$ . Figure 2 shows that when only a single interval (short or long) could be expected during

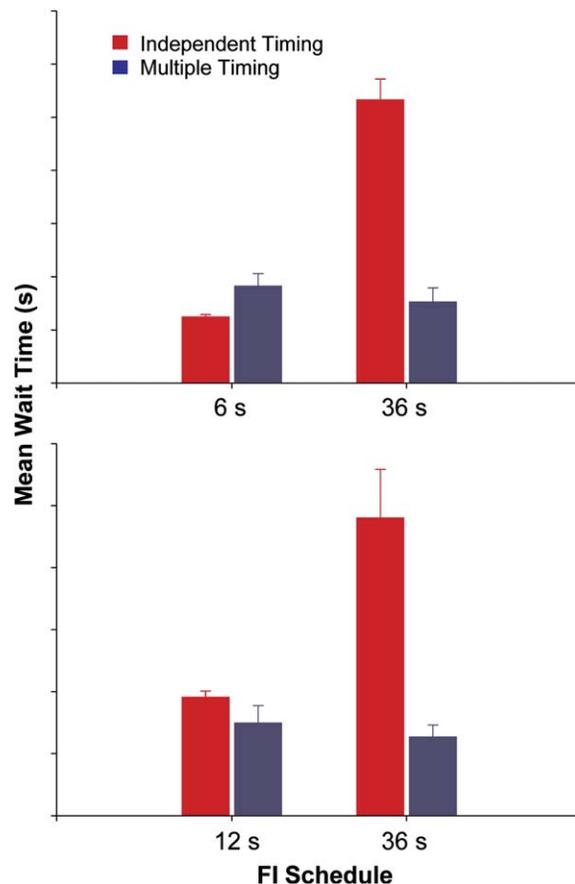


Figure 2. Mean Wait Times for Independent and Multiple Timing Groups

The red bars show data from independent timing groups, and the blue bars show data from multiple timing groups. Wait times were averaged over the last three sessions of training for each group. Error bars are standard errors of the mean.

a session, bumble bees waited until one-third to one-half of the interval had elapsed before making the first response. When two intervals (short and long) could be expected during a session, wait times indicated that bees began by timing the short duration.

Trials in which  $FI_{long}$  is in force are useful for understanding bees' behavior throughout the interval because the multiple timing groups might be expected to time both the short and long components, whereas for the independent timing groups only, the long duration could be in force (Figure 3). Response rates were substantially higher during the first one-third to one-half of the interval for bees in multiple timing conditions than for bees in independent timing conditions. Maximum responding occurred 5 s after the interval began for bees in the multiple timing group that experienced an FI 6 s/FI 36 s combination. Maximum responding occurred over most of the second half of  $FI_{long}$  for bees in the multiple timing group that experienced an FI 12 s/FI 36 s combination. Although neither response function is strictly bimodal, it is clear that both multiple timing groups, and especially M 6/36, were influenced by both the  $FI_{short}$  and the  $FI_{long}$  durations compared to the independent timing groups (Figure 3). Responding was maintained at 75% of the maximum rate over most of the

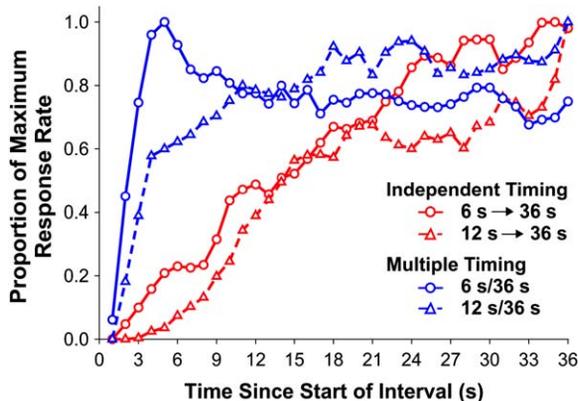


Figure 3. Response Rates during Long Duration (36 s) Trials  
Responding is shown as a proportion of the maximum response rate. The red circles and triangles show data from independent timing groups, and the blue circles and triangles show data from multiple timing groups. Solid lines, FI 6 s/FI 36 s; dashed lines FI 12 s/FI 36.

second half of the interval for both multiple timing groups. Over the second half of the interval, these response functions overlap with those of the two groups experiencing only  $FI_{long}$  at this stage of the experiment. The absence of a prominent peak early in the interval for the group that experienced FI 12 s/FI 36 s suggests that bees had greater difficulty distinguishing these durations than they did 6 s and 36 s.

Patterns of responding within intervals by multiple timing groups were analyzed for trials in which the  $FI_{long}$  was in force. The start of a response burst was defined as the first 1 s time bin containing a response in a string of at least three consecutive bins with a response. The end of a response burst was defined as the first of three consecutive bins that contained no responses after a start. The width of a burst was defined as the difference between the end and the start times, whereas the middle of a burst was the average of the start and end times. The majority of trials in which  $FI_{long}$  was in force were characterized by one or two response bursts. On trials in which there was a single response burst, the burst began on average after  $FI_{short}$  for both multiple timing groups, suggesting that bees were timing  $FI_{long}$  on these trials (FI 6 s/FI 36 s:  $M = 11.50$  s [SEM = 1.76]; FI 12 s/FI 36 s:  $M = 14.31$  s [SEM = 3.17];  $t < 1.0$ ). On trials in which two response bursts occurred, bees' behavior suggested they timed both durations. On such trials, the first response burst by bees trained on the mixed FI 12 s/FI 36 s began later ( $F[1,6] = 8.59$ ,  $p = .026$ ), ended later ( $F[1,6] = 4.57$ ,  $p = 0.076$ ), and had a later midpoint ( $F[1,6] = 8.75$ ,  $p = 0.025$ ) than for bees trained on the mixed FI 6 s/FI 36 s schedule (Figure 4). For both groups, the midpoints of the first response burst was near but after the short interval duration (8.65 s, 13.65 s), suggesting that bumble bees overestimated the short duration, with greater overestimation occurring on FI 6 s.

It is often assumed that insects estimate elapsed durations in many different contexts, including flower-handling times [17], the duration of recruitment dances [18, 19], rates of encounter with nest mates [20], and the integration over time of visual flow [21]. To our knowledge, the findings reported here are the first

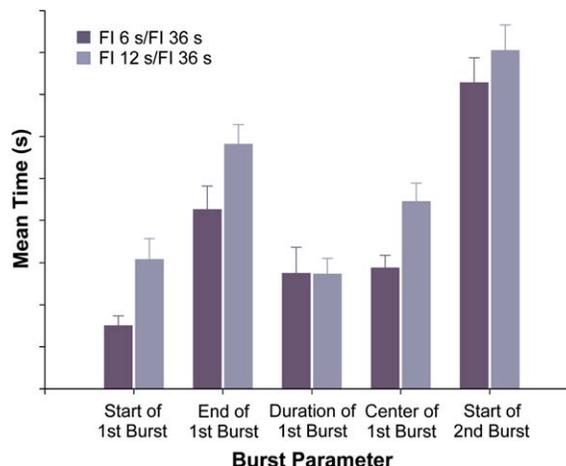


Figure 4. Temporal Properties of Response Bursts by Multiple Timing Groups During Long Duration Trials with Two Bursts

Each parameter is defined in the text. The dark blue bars show data from the group that experienced the FI 6 s/FI 36 s combination, and the light blue bars show data from the group that experienced the FI 12 s/FI 36 s combination. Parameters were averaged over the last three sessions of training for both groups. Error bars are standard errors of the mean.

empirical demonstrations that an insect can time elapsed durations. The results also show that bumble bees can time multiple durations in concurrent fashion. Cognitive and behavioral traits of pollinators influence plant-pollinator interactions and have the potential to influence plant fitness. An important question therefore is whether interval time perception in the range we examined influences foraging patterns in the field. One possibility is that pollinators learn expectations about the intervals, separating successive rewards for specific resources, such as within a particular inflorescence configuration or in a more dispersed patch of flowers, and then compare current estimates of elapsing time with remembered expectations [22]. Elapsing durations that exceed the expected value may cause an individual to switch from the current resource to a different one. Longer interval time perception in the minutes range might function in the measurement of nectar replenishment times. Some avian pollinators adjust their behavior to experimentally imposed replenishment intervals of several minutes in duration [11, 12]. It remains to be determined whether insect pollinators also time intervals in this range associated with nectar replenishment. It has been suggested that interval timing may depend on the neural complexity of the vertebrate forebrain [23], raising the possibility of a phylogenetic divide in interval timing ability. Bumble bees, however, quickly learned to time both single and multiple interval durations associated with food availability. The demonstration of interval timing by bumble bees adds to a growing body of research showing that insects possess cognitive abilities once thought to be restricted to vertebrates. Honeybees can learn contextual information [24], can form categories [25–27], and can learn “same” or “different” rules [28]. Our findings indicate that interval timing abilities are not restricted to vertebrates and may be found not only in other insect pollinators but also in other invertebrates as well.

## Experimental Procedures

### Study Animals and Housing

Female worker bumble bees (*Bombus impatiens*) lived in commercially prepared hives (Biobest Canada Ltd., Leamington, ON), which were housed in the laboratory and provided with pollen daily. Individual workers were uniquely identified with colored nail polish applied to the thorax. Data were collected on 16 bees in this experiment.

### Apparatus

The experiment was conducted in an opaque Plexiglas chamber connected to the colony (Figure 1). Bees entered the chamber through a Plexiglas tunnel. The chamber measured 15 × 7.5 (floor dimensions) × 8 cm (height) and was divided by a wall into two equal-sized compartments. One compartment held the bee, and the other contained apparatus for delivering sucrose reward and for recording the bumble bee's responses. The dividing wall contained a hole located 0.75 cm above the floor. In the chamber, a bee was confined to a space 2.5 × 2.0 (floor dimensions) × 1.5 cm (height) by transparent partitions. An infrared photobeam was mounted behind the dividing wall with ~2 cm separating the emitter and the sensor. Each photobeam component was painted, except for a small hole 1 mm in diameter. This hole narrowed the photobeam sufficiently to permit its interruption when a bee extended its proboscis through the hole in the wall. Each reward was a 3 µl bead of 50% sugar solution delivered by a syringe pump (Sage Instruments, model 341A) to tubing (Clay Adams, PE-90) positioned behind the photobeam mechanism. A bee could collect the reward by extending its proboscis through the hole in the dividing wall until it reached the bead of solution. An interruption of the photobeam occurring after the preprogrammed interval elapsed caused the syringe pump to deliver a reward. The chamber also contained a 24V incandescent light mounted on a side wall adjacent to the dividing wall. A computer and interface controlled the light and syringe pump and recorded each interruption of the photobeam.

### Procedure

#### Pretraining Phase

Each bee was shaped to extend its proboscis through the hole to receive a reward. Bees were then trained for ten sessions with a reward delivered after every response. Each pretraining session began in darkness with a reward available. After consumption of this reward, the chamber light was illuminated. After the next response, the light was extinguished and the syringe pump was activated. This process continued until a bee failed to respond for more than 2 min, at which point it was permitted to return to the colony.

#### Training Phase

Four groups of bumble bees received 30 sessions of 20 intervals per session. One group (n = 4) was trained for 30 sessions of mixed FI 6 s/FI 36 s intervals. A second group (n = 4) was trained for 30 sessions of mixed FI 12 s/FI 36 s intervals. A third group (n = 4) was trained for 15 sessions on FI 6 s intervals, followed by 15 sessions on FI 36 s intervals. A fourth group (n = 4) was trained for 15 sessions on FI 12 s intervals, followed by 15 sessions on FI 36 s intervals. The two components of the mixed interval schedules were equiprobable and randomly intermixed within each session, with the constraint that the shorter FI was always used as the first interval in a session.

Each session began with the chamber light extinguished and a reward available. After consumption of the first reward, the experimenter began the computer program, which turned on the chamber light, recorded responses, and timed events. For the remainder of the session, the chamber light was extinguished and the syringe pump was activated after the first response that occurred after the FI since the last reward elapsed. Bees were given 4 s to consume the reward. After this delay, the light was illuminated and the next interval began. A bee was permitted to return to the colony after the final reward was obtained. Bees completed between one and eight sessions each day, depending on their willingness to enter the chamber.

### Data Analysis

Data were taken from the last three sessions of the experiment to compute wait times, response rate functions, and response burst parameters. Alpha was set at 0.05 for all analyses.

### Authorization for Use of Experimental Animals

All experiments were performed in accordance with the University Council on Animal Care and the Canadian Council on Animal Care guidelines.

### Acknowledgments

We wish to thank W.A. Roberts, K-P. Ossenkopp, and two anonymous reviewers for comments on an earlier version of the manuscript and S. Bamford, F. Boon, J. Ladich, and D. Pulham for technical assistance. This research is part of a doctoral dissertation by M.J.B and was supported by a grant from the Natural Science and Engineering Research Council of Canada to D.F.S.

Received: April 6, 2006

Revised: June 19, 2006

Accepted: June 20, 2006

Published: August 21, 2006

### References

1. Edery, I. (2000). Circadian rhythms in a nutshell. *Physiol. Genomics* 3, 59–74.
2. Mistlberger, R.E. (1994). Circadian food-anticipatory activity – formal models and physiological mechanisms. *Neurosci. Biobehav. Rev.* 18, 171–195.
3. Gallistel, C.R. (1990). *The Organization of Learning* (Cambridge, MA: MIT Press).
4. Richelle, M., and Lejeune, H. (1980). *Time in Animal Behaviour* (New York, NY: Pergamon Press).
5. Meck, W.H., and Church, R.M. (1982). Abstraction of temporal attributes. *Exp. Psychol. Anim. Behav. Process* 8, 226–243.
6. Roberts, W.A., Cheng, K., and Cohen, J.S. (1989). Timing light and tone signals in pigeons. *Exp. Psychol. Anim. Behav. Process* 15, 23–35.
7. Chittka, L., Thomson, J.D., and Waser, N.M. (1999). Flower constancy, insect psychology, and plant evolution. *Naturwissenschaften* 86, 361–377.
8. Osborne, J.L., Clark, S.J., Morris, R.J., Williams, I.H., Riley, J.R., Smith, A.D., Reynolds, D.R., and Edwards, A.S. (1999). A landscape-scale study of bumble bee foraging range and constancy, using harmonic radar. *J. Appl. Ecol.* 36, 519–533.
9. Thomson, J.D., Maddison, W.P., and Plowright, R.C. (1982). Behavior of bumble bee pollinators of *Aralia hispida* Vent. (*Araliaceae*). *Oecologia* 54, 326–336.
10. Waser, N.M. (1986). Flower constancy: definition, cause and measurement. *Am. Nat.* 127, 593–603.
11. Gill, F.B. (1988). Trapline foraging by hermit hummingbirds: Competition for an undefended, renewable resource. *Ecology* 69, 1933–1942.
12. Henderson, J., Hurly, T.A., Bateson, M., and Healy, S.D. (2006). Timing in free-living rufous hummingbirds, *Selasphorus rufus*. *Curr. Biol.* 16, 512–515.
13. Lejeune, H., and Wearden, J.H. (1991). The comparative psychology of fixed-interval responding: some quantitative analyses. *Learn. Motiv.* 22, 84–111.
14. Catania, A.C., and Reynolds, G.S. (1968). A quantitative analysis of responding maintained by interval schedules of reinforcement. *J. Exp. Anal. Behav.* 11, 327–383.
15. Leak, T.M., and Gibbon, J. (1995). Simultaneous timing of multiple intervals: Implications of the scalar property. *Exp. Psychol. Anim. Behav. Process* 21, 3–19.
16. Whitaker, S., Lowe, C.F., and Wearden, J.H. (2003). Multiple-interval timing in rats: Performance on two-valued mixed fixed-interval schedules. *Exp. Psychol. Anim. Behav. Process* 29, 277–291.
17. Ohashi, K. (2002). Consequences of floral complexity for bumblebee-mediated geitonogamous self-pollination in *Salvia nipponica* Miq. (*Labiatae*). *Evolution Int. J. Org. Evolution* 56, 2414–2423.
18. Seeley, T.D., and Tovey, C.A. (1994). Why search time to find a food-storer bee accurately indicates the relative rates of nectar collecting and nectar processing in honey bee colonies. *Anim. Behav.* 47, 311–316.

19. von Frisch, K. (1967). *The Dance Language and Orientation of Bees* (Cambridge, MA: Harvard University Press).
20. Pratt, S.C. (2005). Quorum sensing by encounter rates in the ant *Temnothorax albipennis*. *Behav. Ecol.* *16*, 488–496.
21. Srinivasan, M.V., Zhang, S.W., Altwein, M., and Tautz, J. (2000). Honeybee navigation: nature and calibration of the “odometer”. *Science* *287*, 851–853.
22. Stephens, D.W., and Krebs, J.R. (1986). *Foraging Theory* (Princeton, NJ: Princeton University Press).
23. Hills, T.T. (2003). Toward a unified theory of animal event timing. In *Functional and Neural Mechanisms of Interval Timing*, W.H. Meck, ed. (Boca Raton, FL: CRC Press), pp. 77–112.
24. Collett, T.S., Fauria, K., Dale, K., and Baron, J. (1997). Places and patterns – a study of context learning in honeybees. *J. Comp. Physiol. [A]* *181*, 343–353.
25. Giurfa, M., Eichmann, B., and Menzel, R. (1996). Symmetry perception in an insect. *Nature* *382*, 458–461.
26. van Hateren, J.H., Srinivasan, M.V., and Wait, P.B. (1990). Pattern recognition in bees: orientation discrimination. *J. Comp. Physiol. [A]* *167*, 649–654.
27. Horridge, G.A., and Zhang, S.W. (1994). Pattern vision in honeybees (*Apis mellifera*): flower-like patterns with no predominant orientation. *J. Insect Physiol.* *41*, 681–688.
28. Giurfa, M., Zhang, S.W., Jenett, A., Menzel, R., and Srinivasan, M.V. (2001). The concepts of “sameness” and “difference” in an insect. *Nature* *410*, 930–933.