From foraging to operant conditioning: A new computer-controlled Skinner box to study free-flying nectar gathering behavior in bees

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ABSTRACT

The experimental study of nectar foraging behavior in free-flying bees requires the use of automated devices to control solution delivery and measure dependent variables associated with nectar gathering. We describe a new computer-controlled artificial flower and provide calibration data to measure the precision of the apparatus. Our device is similar to a "Skinner box" and we present data of an experiment where various amounts of a 50% sugar solution are presented randomly to individual bees. These data show large individual variations among subjects across several dependent variables. Finally, we discuss possible applications of our device to problems in behavioral sciences.

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1. Introduction

This paper describes a new computer-controlled artificial flower similar to a "Skinner box." The apparatus is designed for experimental research where various amounts of nectar flow are required. Under natural field conditions, most flowers contain small and variable drops of nectar. For example, 28% of comfrey flowers contain no nectar when sampled in the United Kingdom (Goulson et al., 1998) and the mean nectar volume per flower is only 0.26 μl (Real and Rathcke, 1988; Goulson et al., 2007). Moreover, flower resources are highly variable at the spatial and the temporal level (Leiss and Klinkhamer, 2005; Wolff et al., 2006). As behavior and learning capacities reflect adaptations to the ecological properties of the environment (Stephens, 1993), experimental devices providing small quantities of resources can reveal adaptations in honey bee behavior. This is the rationale behind the development of experimental flowers containing only a few microliters of various solutions.

If drops of sugar solution are delivered manually (Waddington, 1982; Dukas and Real, 1993; Blanschbach, 1994), it is difficult to control the temporal properties of the reward. Moreover, manual presentation of rewards can be a source of unintended error which affects the replication of results. For example, time is especially important in natural flowers resource dynamics. To mimic such a property manually is difficult at best. What is needed is an auto-matic nectar delivery system. We define an automatic flower as a device where the nectar distribution is partially or fully automated via mechanical, electromechanical or electronic command.

Several original devices have been proposed (Ohashi et al., 2006; Makino and Sakai, 2007) but the standard motor driven syringe pump remains the most popular device to deliver nectar (Erber, 1975; Nunez, 1982b; Grosclaude and Nunez, 1998; Paldi et al., 2003; Leadbeater and Chittka, 2008). The syringe pump is often accurate and the amount of flow can be manipulated by modifying the size of the syringe and the speed of the motor. However, as a tool for behavioral research, the standard syringe pump has several limitations. First, the device's electronics do not always provide an accurate flow and calibration may be required (Gray and Smith, 1983). Second, there are often no screws or hardware to connect the pump to other accessories making the pump difficult to integrate with other parts of the apparatus. Third, because the pump is expensive and fragile, it is more often used in laboratory studies than in the field.

In our view, the most important problem limiting the range of behavioral studies comes from the continuous flow. When no bee is consuming the solution, the solution accumulates at the end of the needle and so, the onset and offset of the flow is precisely controlled but consumption is not. To solve this problem, the pump may be manually or electronically activated during a visit and turned off when the crop is filled (Nunez, 1970; Waddington et al., 1981; Nunez, 1982a; Greggers and Menzel, 1993; Schmid-Hempel, 1987; Moffatt and Nunez, 1997; Moffatt, 2001; Naug and Arathi, 2007). However, this kind of solution does not mimic the small drops of nectar that natural flowers provide. Moreover, if the

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nectar flow is higher than the pumping rate of the bee, defined as the volume consumed per unit of time during contact of proboscis with the solution, turning off a syringe pump will still leave drops of sucrose that interferes with the precise control of reward amount during the next visit. Finally, commercial syringe pump do not include software for complex discrete distribution linked to external events.

If nectar is produced continuously by natural flowers, the rate of production is very small and can be less than one micro liter per day (Heinrich, 1976; Real and Rathcke, 1988; Stout and Goulson, 2001). A honey bee visiting natural flowers collects only discrete and small amounts of nectar at each visit. Consequently, discrete distribution devices rather than continuous devices can be used (Grossmann, 1973; Hartling and Plowright, 1979; Sigurdson, 1981a,b; Schmitt and Bertsch, 1990; Keasar, 2000; Tofilski, 2000; Boisvert and Sherry, 2006).

Providing a discrete drop of sugar solution to a honey bee visiting an artificial flower is conceptually similar to delivering a food pellet to a rat when pressing a lever in a Skinner box and can be taken as an example of instrumental or operant conditioning (Abramson, 1994). In this way, a flower can be conceived as a natural operant conditioning chamber and the artificial flower a controlled scientific tool designed to study the effect of reinforcement on honey bee choice and behavior. The analogy between foraging and conditioning has been recognized for many years (Shettleworth, 1989).

Often, environmental constraints involved during foraging can be simulated with standard reinforcement schedules. For example, variable ratio or interval schedules mimic reinforcement distribution after random flower visitation by honey bees. Patch depletion is also equivalent to progressive schedules with time or response requirement increasing with successive reinforcers. However, despite many papers published on honey bee foraging behavior, such procedures have never been systemically used until recently. Many experimenters use only continuous reinforcement schedules with small and constant reinforcer amounts or with continuous delivery until the crop is filled (Nunez, 1970, 1982a; Schmitt and Bertsch, 1990; Greggers and Menzel, 1993; Tofilski, 2000; Moffatt, 2001).

To this date, only four studies have collected data with intermittent reinforcement schedules (Grossmann, 1973; Sigurdson, 1981a,b; Boisvert and Sherry, 2006). None of these studies explored systematically schedule controlled behavior or used the device to test predictions from optimal foraging models or measure physiological parameters.

2. Method

2.1. A new computer controlled and ecologically inspired artificial flower/operant conditioning chamber

The first step in designing an operant conditioning chamber for honey bees is to define the operant response that will provide access to the reinforcer. Some authors reinforced proboscis extension either with infrared, capacitive or oscillator-frequency detection (Sigurdson, 1981b; Schmitt and Bertsch, 1990; Fulop and Menzel, 2000; Boisvert and Sherry, 2006). In this case, the energetic cost of the response is very low and, consequently, high response rates were observed. The problem of using proboscis extension as an operant response is the fact that response cost cannot be varied to a large extend. This could be a problem for ecological inspired studies which are based, in part, on the fact that a honey bee has to travel long distances and visit large number of flowers to fill her crop (Seeley, 1995). With the choice of hole entering defining the operant, the length of the hole can be adjusted thereby mimicking the complexity of a natural flower.

Based upon the issues presented in the previous section we decided to use entering a hole as the operant response. A 6 mm diameter hole drilled in a 36 mm plastic platform (the honey bee platform) provides access to a response hole connected to the cone of a standard luer orange needle (0.5 mm external diameter, 0.24 internal diameter). The needle is cut and ground close to the cone providing a flat surface for solution distribution. The small size of the needle hole prevents evaporation inside the needle. A small aluminum 5 mm diameter cup is glued to the cone to avoid the accumulation of sucrose and prevent the solution moving along the cone. Fig. 1 shows details of the response hole and access to the needle. Moving in and out of the hole is detected with one infrared emitter and detector pair (Honeywell SEP8736 and SDP8436) connected to standard electronic components soldered onto a printed 38 mm × 145 mm circuit board located near the response hole. The honey bee platform is easily removed for inspection, cleaning and shaping. It is positioned inside of a 20 mm diameter hole made in a white polycarbonate 33 cm × 40 cm plate. The size of the response hole has been adapted to work with European honey bees, however, the size is easily changed for bigger (for example bumblebees) or smaller insects species (for example some species of flies).

The reinforcer is any type of liquid and is stored in a 3 ml glass syringe. The syringe is locked in place with an aluminum part and screw. The piston is pushed by a steel plate moved by a 200 steps motor with two gear wheels and a 2 mm trapezoidal screw. The linear movement of the steel plate is obtained with two 8 mm brass tubes guiding the piece in transition. The length of the two brass tubes exceeds the length of the apparatus and ends with a screw thread. The two screws shown on Fig. 1 can be used to fix several accessories to the solution dispenser (for example, the printed circuit board or the landing platform). With a 3 ml syringe, each motor step corresponds to 0.15 μl. As shown in Fig. 1, the axis of the syringe exactly superposes the axis of the response hole. Because the step motor is activated only if a response is detected, this protocol prevents solution accumulation and evaporation in the cup.

Two standard micro switches are used as end position detectors to detect extreme positions of the steel plate to avoid the syringe or gear breaking. The step motor interface is placed under the feeder and protected against spillage with a plastic transparent plate. To obtain better control and precision of solution flow and reinforcer amount (Gray and Smith, 1983), we used a simple transistor circuit to control the motion of the step motor. The timing parameters of step motor rotation are defined by the software. Moreover, the step motor was locked in position between two successive uses to ensure it would stay in position. After several tests, it appeared that only glass syringes provided a constant amount of solution. A standard plastic syringe can compress and our tests showed an irregular distribution of sucrose solution even with continuous motor rotation.

When conditioned free-flying honey bees failed to find a drop of solution inside the response hole they often flew around the artificial flower to gain access to the feeder from the underside of the landing platform. To prevent such "robber" behavior, and to avoid crushing a trained honey bee with the turning gears, we enclosed the mechanical and electronic parts of the device within a rectangular 21 cm × 21.5 cm × 30 cm polycarbonate white box (width × depth × height). A 4 cm × 4 cm 12 V fan was placed inside the box to cool the electronic components. So that the experimenter can gain easy access to the syringe, a 21.5 cm × 27.5 cm transparent Plexiglas door was constructed.

A wooden table (42 cm × 34 cm × 117 cm) adjusted to the apparatus size was built to put the landing platform in a horizontal position (the syringe is vertical). The small size and weight of the table allowed the experimenter to use the experimental chamber in the field. Fig. 2 shows the wooden table with the automated
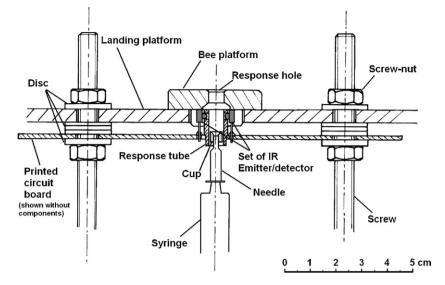
Fig. 1. Schematic diagram of the syringe and bee compartment. The cut out view is shown with oblique lines. The electronic components soldered on the printed circuit board are not shown.

artificial flower and the landing platform. To easily manipulate the device and position it on the table, two steel handles were screwed on both sides of the landing platform. A manually activated robotic cover was also fixed on the landing platform to close the response hole between experimental phases.

To restrict access to the operant chamber to an individual honey bee, we built a Plexiglas chamber that attached to the landing platform. Fig. 2 shows the chamber. The dimensions
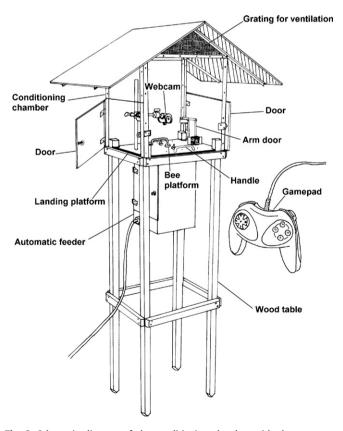

Fig. 2. Schematic diagram of the conditioning chamber with the computer-controlled artificial flower including the webcam, table, two-door chamber and gamepad (the gamepad has an exaggerated size due to perspective).

are 44 cm × 35 cm × 51 cm with an internal volume of 69 l. These dimensions are sufficient for the honey bee to fly within the chamber. Two 37.5 cm × 20 cm doors are placed on opposite sides of the chamber.

To limit light falling upon the landing platform that might disrupt the photocells used to detect the operant response, the back and roof of the Plexiglas chamber is covered with cardboard. The upper part of the chamber had slots to increase air circulation. We detected no particular problem to use the doors with several marked honey bees. When a honey bee arrived, she waited in front of one door, turning sometimes around the box or landing on the door until the other honey bee, inside the chamber, left. The honey bee waiting outside immediately entered the chamber. When two bees tried to enter simultaneously, a small aquarium fishnet was used to discard one bee without inducing notable stress. An USB gamepad (Thrustmaster digital 3) was used as a keyboard to select the visiting bee, each bee having her own button.

Our device comes equipped to record video from a high resolution USB webcam. A 30 cm long vertical aluminum tube is fixed to the landing platform 16 cm from the response hole. An adjustable nut secures a Philips SPC 900NC webcam above the response hole. This model was selected because it has the necessary hardware to secure the camera to a fixed position and can capture images with 640 pixels × 480 pixels resolution with 90 frames/s. The webcam can be controlled by software when a honey bee is detected or operated manually. Images are all stored with a unique identifier for rapid identification.

The device is connected to the computer with a 4-m wire via a control unit. This unit is equipped with power (5 and 12 V), a cooling fan and an USB Input/output circuit board (Code Mercenaries IO-Warrior40). Four independent artificial flowers can be connected to the unit.

2.2. The control software

The behavioral control software is written with Borland Delphi 6 and functions on a PC computer (Windows 98 or XP). The software has many features and only the most important will be discussed. Timing functions provides millisecond precision and come from the port.dll file (Kainka, 1999). During a response, the state of the infrared detector may oscillate when a honey bee moved inside the hole and several responses may be recorded.

A debouncing algorithm eliminated false responses. When the program detected an IR sensor state change inside the response hole, it is recognized as a response event only after a defined period of time (a value of 100 ms in most cases).

We incorporated into the software a feature to precisely control reinforcement delivery. Consider a situation where a large reinforcer is not completely consumed. Some of the residual nectar or sucrose solution will be added to the next reinforcer thereby introducing uncontrolled variation. To avoid this problem, the distribution of the reinforcer follows "bursts" of drops, the number of drops and the inter bursts interval being defined in a parameter window. Moreover, if a bee leaves the hole before the end of a distribution, the motor driving the syringe stops automatically.

The software can work simultaneously with four marked honey bees. Any event during a session can be monitored in real time and observed on the computer screen with various graphical or text symbols. Events are stored in a main data file and coded as follows: time, state of event (beginning or end), type of event (for example a response or reinforcer), number of event (for example response number 3), intensity of the event and subject number to which the event is applied.

The session manager is the main window of the software. Here we define the length of a session (constant length, or constant amount), the number of experimental conditions and the schedule parameters (ratio or interval schedule) for each experimental honey bee. Once the session begins, the window displays information about reinforcement number, volume consumed, number of responses, number of visits, and detailed information about the progress of an individual honey bee in meeting the requirements of the reinforcement schedule such as time and number of responses since the last reinforcement. At this date, the session manager can run fixed and variable interval and ratio schedules.

For each honey bee, the software measured several dependent variables: response length (time spent in the response tube), inter-response interval, visit length (time between the beginning of the first response and the end of the last response of a visit), inter-visit interval (includes time spent flying to the hive to unload and returning to the conditioning chamber), number of responses, number of reinforcers, response rate, and load size per visit, inter-reinforcer interval, inter-reinforcer response number and inter-reinforcer response rate. A list of text files containing these data is created in real time and the experimenter can follow the evolution of the measures on a list of 10 graphic windows. Special attention can be given to the cumulative curve that appears in the middle of the session manager. At the end of the session, the software automatically saved all files in separate folders and builds a pdf file containing the session data and graphics for rapid examination. Finally, when a session terminated, the software automatically returns the syringe to its starting position for cleaning.

2.3. Calibration experiment

As mentioned earlier, commercially available syringe pumps can experience severe precision problems (Gray and Smith, 1983). Therefore it is important to collect calibration data and we present such data on our device. Several technical solutions have been proposed to test the precision of syringe pumps (Nieman et al., 1986). We programmed various fixed amounts of solution, 0.45, 0.61, 0.76, 0.91, 1.06, 1.51, 1.97, 3.03, 3.94 and 4.54 μl, corresponding to 3, 4, 5, 6, 7, 10, 13, 20, 26 and 30 motor steps (each step corresponding to 0.15 μl) and measured the corresponding volume of solution stored in the cup with a 5 ml capillary tube. Two sugar concentrations were tested, 50% and 30%. Each volume was repeated five times and the cup cleaned after each delivery. The amount of sucrose in the tube was evaluated and averaged by two independent experimenters.

2.4. Test experiment

The experiment was performed in Stillwater, Oklahoma, during November 2008. The purpose of the experiment was to test the apparatus and software. In addition, we wanted to observe the behavior of honey bees inside the device and to collect sample data about the rate that honey bees collect sucrose solution.

2.4.1. Subjects

The subjects were European honey bees from 6 different colonies. However, for the experiment, only 4 honey bees were used.

2.4.2. Apparatus

The conditioning chamber was located in a garden at about 25 m from the hives. The control box and the computer (portable PC computer, Windows XP) were located inside a small garage 3 m from the chamber. Approximately midway between the hives and the conditioning chamber, a feeder was continuously providing a 10% sugar solution. The feeder was established to attract foragers that would be subsequently used as experimental subjects.

2.4.3. Procedure

2.4.3.1. Recruitment and shaping phase. When a large number of honey bees began to regularly visit the feeder, we placed a few drops of 50% sucrose solution in a Petri dish and forced one or two bees to consume the drops. While they were feeding on the solution, we slowly moved the Petri dish inside of the conditioning chamber and placed several more drops on the landing platform. Once they filled their crops, the bees would often return to the conditioning chamber and actively started to search for food. If they did not return to the conditioning chamber we could find them on the feeder where they were again captured and placed on the landing platform. After few minutes, we observed some recruitment and about one dozen honey bees landed on the platform. We then started the shaping phase by placing some new drops of solution closer and closer to the response hole

When the honey bees were close to the response hole we deposited more drops around the borderline of the hole and some inside the hole. This shaping method is sufficient to stimulate at least one honey bee to enter the response hole. During the shaping phase, all hole entering responses were reinforced with a 5 μl drop of 50% sugar solution. When several bees were regularly entering the hole, we stopped depositing drops on the landing platform and started to mark the honey bees for identification.

2.4.3.2. Marking bees. We marked four bees with standard beekeeping color and number tags (Betterbee queen number set). We used only one number per color to be able to visually identify all of the subjects. We also used a standard bee marking cage placed on the platform when a honey bee was inside the response hole. The four marked honey bees were white 18 (W18), red 13 (R13), blue 6 (B6) and green 12 (G12). Once the marked honey bees were returning to the conditioning chamber regularly, we used the doors of the conditioning chamber to restrict access to only a single marked honey bee and the experiment began

2.4.3.3. Experimental design. All honey bees worked simultaneously but only one was allowed to fill its crop at a time. The experimenter detected the arriving bee and used the gamepad to send subject information to the computer. All honey bees were submitted to the same experimental conditions, a fixed ratio 2 schedule with a reinforcer amount chosen randomly from the following values with the restriction that no value was given on two consecutive occasions: 0.90, 2.55, 4.19, 5.84 or 7.34 μl of 50% sugar solution. The software bounce protection delay was set to 100 ms, and the
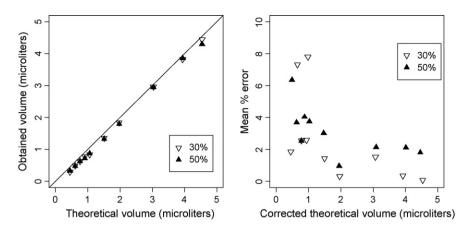
Fig. 3. (Left panel) Obtained volume (μl) as a function of theoretical volumes (μl, based on the expected volumes delivered by the step motor). The strait line ($Y = X$) shows the perfect equality. (Right panel) Relative error (percentage of measure) as a function of corrected theoretical volumes (see text). For both graphs, the black triangles correspond to the 50% sugar solution and the white triangles to the 30% solution.

post-reinforcer time set to 200 ms. The experiment ended after two hours of data gathering, each bee allowed to visit the flower five to six times

3. Results

3.1. Calibration experiment

The left panel of Fig. 3 shows the obtained volumes is a function of the expected quantity. As predicted, for both concentrations, we obtained a linear relationship close to perfect equality. Our device provides good control over the amount of sucrose solution. Linear regression analysis gives $Y = 1.015x - 0.166$ ($R^2 = 0.999$) for the 30% solution and $Y = 0.998x - 0.150$ ($R^2 = 0.999$) for the 50% solution. This analysis reveals that the amount of sucrose given is a linear function of the expected quantity (slopes very close to one), but a little bit smaller than the quantity expected (negative intercept). This quantity is close to 0.15 ml, the volume corresponding to one motor step. Such a shift could come from the measurement technique (capillary tubes). With the high viscosity of the sugar solution, we might expect a small amount of solution remaining in the bottom of the cup. Another possibility is the cleaning method. We used cotton swaps after each delivery. The shift could also be the consequence of some small gap around the trapezoidal screw driving the syringe piston. The cause of this error is unknown at the present time and would require additional tests.

Because the relationship between the expected quantity and the obtained quantity is well known we can use the two linear equations to correct the prediction of the obtained volumes. We did this and computed the mean percentage error, that is, the error of delivery divided by the expected corrected volume × 100.

The right panel of Fig. 3 shows the relative error for each corrected volume delivered. Of course, because the shift is constant across volumes, we expect a decreasing relationship. We obtained precisely this relation. The error never exceeds 8%, falls to 4% for volumes greater than 1 μl, and smaller than 2% for volumes between 2 and 5 μl. Because the artificial flower is made with very simple and inexpensive electronics and mechanics, we consider the precision of the device to be good for most purposes.

3.2. Test experiment

The honey bees took less than one hour to visit the conditioning chamber five to six times. If a honey bee found the door of the chamber closed (because the chamber was being used by another honey bee) it would fly around the chamber or the experimenter

until the door opened. When the door opened the honey bee inside the chamber would fly away to unload and the honey bee waiting outside the chamber entered. During the session, no bee returned to the hive before filling its crop. Moreover, no bee left the apparatus to forage in another patch.

Fig. 4 shows the main behavioral measures for the four bees. Because we used a fixed ratio 2 schedule of reinforcement, it is possible to measure inter-response times (IRTs). An IRT is defined as the time between the end of a response and the beginning of the next response. Of course, when the honey bee returns to the hive a long IRT is recorded and such data is removed from the analysis.

Panel A of Fig. 4 shows the mean IRTs for the four honey bees. The mean value of the IRT ranges from 0.5 to 2.2 s. One honey bee, bee W18, responded with longer IRTs. We used the Wilcoxon rank test (Dalgaard, 2008) to compare all pairs of honey bees. Only the difference between bee W18 and bees B6/G12 were significant ($p < 0.05$).

Panel B shows the mean visit length defined as the time between the beginning of the first response of a visit and the end of last response before returning to the hive. As this Panel shows, the honey bees stayed around 150 s in the apparatus before departing. Bee B6 stayed a little longer in the apparatus but only differences between bee B6 and bees R13/G12 were significant (Wilcoxon rank test, $p < 0.05$).

Panel C shows that the load size remained approximately constant among honey bees (means between 47.30 and 50.22 μl). No significant differences among the honey bees were detected (Wicoxon rank test, $p > 0.05$). The values fall within the typical ranges found in honey bees, the maximal crop load being around 60 μl (Nunez, 1982a; Varju and Nunez, 1991).

Panel D shows data on nectar flow. Load size divided by visit length defined the solution flow in the chamber. This flow was between 17.7 and 22 μl/min and no significant differences among the honey bees were detected (Wilcoxon rank test, $p > 0.05$). This value can be used to compare the load size obtained with continuous flows provided by standard syringe pump systems. For example, an asymptotic increasing load size with an increasing flow rate is a well known phenomenon (Nunez, 1982a; Varju and Nunez, 1991; Moffatt and Nunez, 1997), the maximal value being reached with 50% sugar solution for flow values range from 5 to 15 μl/min. The observed load sizes in this experiment were consistent with previous data.

Panel E shows data on mean inter-reinforcer intervals (time between the end of a reinforced response and the beginning of the next reinforced response). The mean inter-reinforcer interval differs from IRTs because it includes response length. It is interesting to note that bee W18 had longer IRTs (panel A), but had the
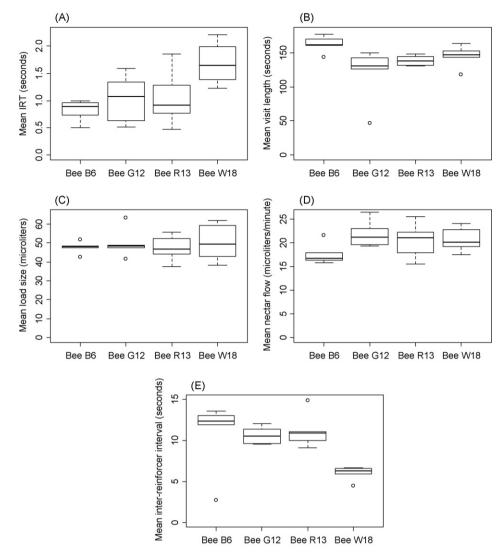
Fig. 4. Box plots of individual bee dependent variables (panel A: mean IRT, panel B: mean visit length, panel C: mean load size, panel D: mean local nectar flow and panel E: mean inter-reinforcer interval). The extreme values of the vertical bars show lowest and highest values. The first and third quartiles are shown with bar limits and the median values correspond to the thick horizontal line inside the bar. Dots are identified as outliers.

shortest mean inter-reinforcer intervals. The Wilcoxon rank test revealed only two significant differences between W18 and bees R13/G12 ($p < 0.05$). This apparent contradiction can be understood if we noticed that honey bee W18 had one of the highest pumping rates and the fastest times to exit the response hole (see next section).

The experimental design provided the opportunity to precisely measure the pumping rate from the response length data. Fig. 5 shows the individual relationship between the amount of sucrose solution received and the time spent in the response hole. For all four bees, a linear relationship described the data very well. All R^2 values are greater than 0.986 and all slopes differed significantly from zero ($p < 0.001$). Note that for all bees, residuals distributed normally around regression lines (Lilliefors test, $p > 0.05$). As indicated in Table 1, the values of the slopes ranged from 1.17 to 1.57. For all honey bees, we observed positive intercepts that differed significantly from zero ($p < 0.01$). The values ranged between 1.66 and 2.8 s and corresponded to time spent in the hole without consuming the solution.

To go in and out from the tube takes some time and we can consider this variable as a measure of flower handling time. A longer response hole would probably correspond to greater intercepts. To compare the four bees, covariance analyses for all pairs of bees can

be done. However, we need first to show that the variances around regression lines are the same for all the pairs. As the variance can be considered different for B6 and G12 ($p < 0.05$), no comparison of slope and intercept has been done for these two bees. As suggested by Fig. 5, we observe two bees with short intercepts (R13 and W18) and two with long intercepts (B6 and G12). No difference in intercept is significant in the short intercept group ($p > 0.05$), but differences are significant for all between-subgroup pairs of bees ($p < 0.05$). B6 had the higher slope and the difference is significant with all other bees ($p < 0.01$). No differences in slope between R13, G12 and W18 were significant ($p > 0.05$).

The solution pumping rate can be obtained from data in Table 1. It is simply computed from the reverse slope (multiplied by a factor 60 to change the time scale to minutes). The rate values range

Table 1
Summary of response length measures for all four bees from Fig. 5. For pumping rate calculation, see text.

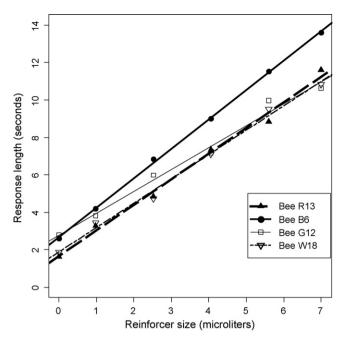|	Bee R13	Bee B6	Bee G12	Bee W18
Slope	1.37	1.57	1.17	1.3
Intercept	1.66	2.7	2.8	1.88
R^2	0.992	0.999	0.986	0.993
Pumping rate (μl/min)	43.89	38.25	51.36	46.21

Fig. 5. Response length as a function of reinforcer size for individual bees. The black triangles are data for bee R13, the black circles data for bee B6, the white squares data for bee G12 and the white triangles, data for bee W18. Regression lines are plotted separately for each bee (thick interrupted line: bee R13, thick continuous line: bee B6, thin line: bee G12 and thin interrupted line: bee W18). See text and Table 1 for equations.

between 38.25 and 51.36 μl/min. These values are comparable with previous 50% sugar solution studies (Nunez, 1970) and a little bit smaller than values observed with less concentred solutions (Fulop and Menzel, 2000).

4. Discussion

Bees have no particular problem visiting and gathering the solution provided by our device. Before running the experiment, the experimenter spent dozen of hours simulating visits with a pencil to test all the software options and data gathering procedures. Neither the hardware nor the software seems to suffer from hidden weaknesses. We obtained data similar to what is known about crop load or learning with comparable devices (Sigurdson, 1981a,b; Nunez, 1982a; Fulop and Menzel, 2000) and consequently, our device has proved to be a good apparatus to study the nectar foraging behavior of individual free-flying honey bees. Moreover, the calibration data give us a correction formula for distributed volumes and information about the precision of the apparatus. However, this precision can be achieved only if the volume of the stored solution inside the syringe does not vary with temperature. As honey bees collected reinforcers during high rate visits, the temperature variations between two visits varied only in a very small range. Consequently, volume variation due to temperature remained at a very low level.

In the test experiment, we have been able to show individual differences in some parameters of foraging behavior. Contrary to Fulop and Menzel (2000), we observed inter-individual differences in pumping behavior. Our result suggests that calibration of Fulop and Menzel's apparatus based on mean bee visit length is inadequate to assess the given reinforcer amount and that direct technical control of reinforcer amount is a better solution for experimental control of nectar drop properties. Such inter-individual differences should require special attention in the future because it has been shown that honey bee task partitioning may be linked to individual behav-

ioral variation (Scheiner et al., 2004; Roussel et al., 2009). Because our device automatically provides data on a wide range of independent variables we suggest that it can significantly contribute to the identification and description of phenotypic variation.

The complete automation of experimental protocols now opens the possibility to easily perform experiments in the laboratory or in the field while measuring a complete set of parameters related to individual behavior. Several scientific fields should benefit from our device. First, as shown with the data concerning pumping rate, the device could be used to measure physiological parameters (Moffatt and Nunez, 1997; Moffatt, 2001). Secondly, the automatic flower offers interesting perspectives in the context of optimal foraging models. The experimental test of this kind of quantitative model requires known and controlled resource properties (for example patch depletion). As natural flower resources are generally unknown, it is often difficult to test these models in the field. Software controlled flowers open the door to unlimited number of protocols and reinforcement schedules are one example of such protocols. The fact that the device provide discrete amount of solution and a natural flower a continuous nectar flow does not constitute a limitation to ecological applications because a continuous flow can be approximated with a discrete set of values. Thirdly, the device can be used as a Skinner box for honey bees, thereby opening the way to new investigations into comparative studies of honey bee learning abilities. Moreover, even though the apparatus was designed for honey bees it can easily accommodate larger or smaller bees including bumble bees. Finally, because the device not only provides measures of foraging behavior, but also controls access to a consumed solution, the device offers interesting perspectives in toxicology studies.

Our device is different from other automatic flowers because it combines a unique set of characteristics. All the components are assembled together as an integrated USB device and the researcher can use the device in the laboratory or in the field. As solution distribution is contingent on visits, it does not accumulate between visits. As a consequence, we have good control of solution amount collected by honey bees. Another important characteristic of the device is the delivery of discrete amount – fixed or variable – of reinforcers under computer control.

Our apparatus differs from the Grossmann (1973) and Sigurdson (1981a, 1981b) devices in several respects. These devices implemented the experimental procedure from hardware and not from software. As a consequence, the reinforcer amount could not vary across conditions and only a limited number of standard reinforcement schedules could be used (fixed interval and fixed and variable ratio). Second, they could work with only one bee at a time. Our software can collect individual data about four different marked bees. Moreover, a complete set of dependent variables is automatically recorded for each bee. Only response rate was collected by Grossmann (1973) or Sigurdson (1981a, 1981b). Finally, these designs were not intended for the study of optimal foraging models or the measurement of physiological parameters. Because the software is written in a popular programming language, it will be possible in the future to modify it to implement new protocols in the analysis of learning and/or foraging behavior.

Several technical questions still need to be addressed. First, during crop filling, the honey bee is inside a closed conditioning chamber. It is unknown how such confinement affects nectar gathering at the source. For example, Moffatt and Nunez (1997) showed that confinement in a small respirometric chamber affects crop load and visit and inter-visit length. Their chamber was smaller than the one we used. In our chamber, the honey bee is free to fly and turn around on the bee platform before landing and she can do this several times before the end of crop filling. Such behavior suggests our chamber will have less of an effect that the chamber used by Moffat and Nunez. Second, we used only small reinforcer amounts (less

than 7 μl). These small drops are completely consumed. However, it is probable than larger drops would not be fully pumped especially at the end of crop filling. To deal with this problem, the software can distribute the overall reinforcer amount with bursts of drops with an inter drop interval, this interval to be used to pump the solution. At the end of each interval, the bee could remain inside the tube and wait a few milliseconds for the next burst, or return to the hive after filling its crop. In the latter case, the series of bursts would immediately stop and prevent the solution accumulation in the cup. Such a protocol has not been tested here and bursts parameters need still to be defined.

At this time, several automatic flowers have been built but the software can presently control only one at a time. Our next step will include software development for choice studies using several devices simultaneously (Sigurdson, 1981a,b). A 3-color stimulus module will also be added to study stimulus effects (Sigurdson, 1981a,b). Another extension module is under study, an automatic tag detection device linked to an automatic door. This extension would allow fully automated experiments without experimenter intervention. Finally, an autonomous version of the device using a microcontroller is also under development.

Laboratory studies of learning have often been criticized because of the artificial dimension of space, stimuli, reinforcers and motivational conditions (Houston, 2009). For example, because it has been shown that the environment scale may affect optimal behavior (Ranta et al., 2000), the use of laboratory situations to study behavioral rules and decision mechanisms must be applied carefully. In that perspective, our bee conditioning chamber offers original characteristics. Contrary to all conventional conditioning chambers, the animal decides when to visit and to leave the device. Consequently, even if this point should be examined carefully, all other aspects of honey bee life, especially social behaviors (communication, prophylaxis, etc.), may remain unchanged. Moreover, the response topography of tube entering resembles the natural responses emitted by bees with real flowers. For all these reasons, we believe that our device has high ecological validity and is well adapted to discover behavioral rules that have been shaped in the past by natural selection.

Acknowledgements

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