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— Short Note on Methodology —

FLOBOTS: ROBOTIC FLOWERS FOR BEE BEHAVIOUR EXPERIMENTS

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Abstract—Studies of pollinator foraging behaviour often require artificial flowers that can refill themselves, allowing pollinators to forage for long periods of time under experimental conditions. Here I describe a design for inexpensive flowers that can refill themselves upon demand and that are easy enough to set up and clean that they can be used in arrays of 30 or more flowers. I also summarize of a variety of artificial flower designs developed by other researchers.

Keywords: artificial flowers, pollinator behaviour, nectar, learning, cognition, methods

INTRODUCTION

Studies of pollinator foraging behaviour have played an important role in furthering our understanding of plant-pollinator interactions (e.g., Schmitt 1980; Chittka et al. 1999; Maloof & Inouye 2000). Artificial flowers are often useful in foraging studies because of the degree of control they allow over variables such as floral cues, nectar sugar concentrations, and timing of reward provision. In some cases a researcher can simply refill flowers manually as they are emptied (e.g., Gegear & Laverty 2005). However, in experiments in which floral rewards vary in volume or composition or in which the experimental array occupies a large area, this approach can easily become impractical. In these cases, a substantial amount of time may need to be spent refilling flowers between the subject's foraging bouts. Furthermore, when large numbers of flowers are used, the water in the sugar syrup can evaporate away before it is consumed.

Researchers have designed a variety of self-refilling artificial flowers to solve these problems, each with its own advantages and disadvantages (Tab. 1). A particularly inexpensive and versatile design is described by Makino and Sakai (2007) and further developed by Thomson et al. (2012). In these flowers, a thin wick carries sugar solution from a reservoir into a flower. The flowers are simple and inexpensive to construct and can run for long periods of time. However, the rate of refilling cannot easily be controlled.

A variety of other designs do offer control over refilling rate, although they are more expensive and difficult to construct. The most common approach is to use syringes, attached to pumps or step motors, to supply sugar solution to flowers (Tab. 1). If tubes from two different pumps, supplying different concentrations of sugar solution, feed a single flower, the concentration of sugar syrup in the flower can be varied by adjusting the volumes provided by each pump (Nachev & Winter 2012). Ohashi et al. (2010) use a motor to lift one end of a length of flexible tubing containing sugar solution, forcing the solution to flow out of the other end into artificial flowers. In Keasar's (2000) artificial flowers, a shallow cup, attached to the top of a buoyant, metal-bottomed cylinder floating in a reservoir of sugar solution, can be dipped into the reservoir by an electromagnet located under the reservoir (see also Cnaani et al. 2006; Lihoreau et al. 2010). Hartling and Plowright's (1979) artificial flower consists of a capillary tube that is dipped into a reservoir by a combination of an electromagnet (which pulls down a metal arm attached to the capillary tube) and a spring (which pulls the arm back up). Fülöp and Menzel (2000) use a motor attached to an eccentric cam to dip a lever into sugar syrup, lift it into an area accessible to the bee, and then, a set period of time after the bee's proboscis comes into contact with it, move it back out of reach.

Most automatically-refilling flowers are designed for arrays of only a few flowers, and both the expense required to build them and the time required to set up and clean each flower could pose problems if a researcher wished to build an array with many flowers (although see Keasar 2000; Paldi et al. 2003; Winter & Stich 2005; Ohashi et al. 2013). Here I describe a new design suitable for use in arrays of 30 or more flowers. In these flowers, a metal rod is dipped into a reservoir, lifting out a small droplet of sugar solution. The volume of the droplet varies, but the design does allow precise control over the timing of reward delivery.

Although these flowers have two important disadvantages over most of the designs described above, namely the lack of control over nectar volume and the possibility that refilling flowers will startle nearby bees, they also have a number of compensating advantages. The flowers can be set up, disassembled, and cleaned quickly

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Table 1. Summary of artificial flower designs capable of automatic refilling.

Delivery system	Citations	Delivery type	Can control timing of delivery	Can control volume delivered
Wick connected to a reservoir	Makino and Sakai 2007; Thomson et al. 2012	Continuous	No	Partly*
Flexible tubing lifted by a motor	Ohashi et al. 2010	Continuous	Yes	Yes
Scholander microburette driven by a motor	Núñez 1971, Giurfa and Núñez 1992; Moffatt 2001	Continuous	Yes	Yes
Syringe pump†	Paldi et al. 2003; Naug & Arathi 2007; Ings & Chittka 2008	Continuous	Yes	Yes
Syringe connected to a stepper motor or pump	Grossman 1973; Sigurdson 1981; Waddington et al. 1981; Schmitt & Bertsch 1990; Greggers and Menzel 1993; Tofilski 2000; Boisvert and Sherry 2006; Sokolowski & Abramson 2010	Discrete	Yes	Yes‡
Syringe pump and pinch valves regulating flow of solution through tubes into flowers	Winter and Stich 2005; Nachev and Winter 2012	Discrete	Yes	Yes
Miniature solenoid valve releasing solution on demand	Brown and Gass 1993	Discrete	Yes	Yes
Cup on a float pulled down into a reservoir by an electromagnet	Keasar 2000; Cnaani et al. 2006; Lihoreau et al. 2010	Discrete	Yes	Yes
Lever dipped into a reservoir by a motor-driven eccentric cam and removed from reach a set time after bee begins drinking	Fülöp and Menzel 2000	Discrete	Yes	Controls duration of access
Capillary tube dipped into a reservoir by a lever attached to an electromagnet	Hartling and Plowright 1979	Discrete	Yes	Yes
Brass rod dipped into a reservoir by a solenoid	This paper	Discrete	Yes	No

*Although precise control over volume cannot be achieved, the relative volume delivered can be controlled by varying the number (or size) of knots or the number of wicks in a flower (T. Makino and J. Thomson, personal communication).

†Peristaltic pumps are a less expensive alternative, although they deliver more variable volumes than syringe pumps (Y. Winter, personal communication).

‡The level of control over volume varies across these designs. The Greggers and Menzel (1993) design delivers the highest degree of precision (±0.02μL).

enough to make the use of a large array feasible, and the components are inexpensive enough to allow construction of such an array on a small budget. Reservoirs and dipping elements can be quickly and easily replaced or swapped between flowers, facilitating changes in the distribution of rewards across flowers during the course of an experiment. Changing which flowers are rewarding reduces the bees' ability to use cues not intended by the researcher, such as scent marks or spatial cues (Church & Plowright 2006; Goulson 2009), to identify rewarding flowers. Finally, the construction requires only a few, common tools, such as a drill press and soldering iron, and the design is robust to both imperfections in construction and damage during use.

MATERIALS AND METHODS

Flobot design

Each flower consists of an enclosed reservoir and a 1.6 mm-diameter brass rod that is dipped into the reservoir, lifting out a small droplet of liquid (which adheres to the sides of the rod) and making it accessible to foragers (Fig. 1). The brass rod is lowered into the reservoir by a solenoid

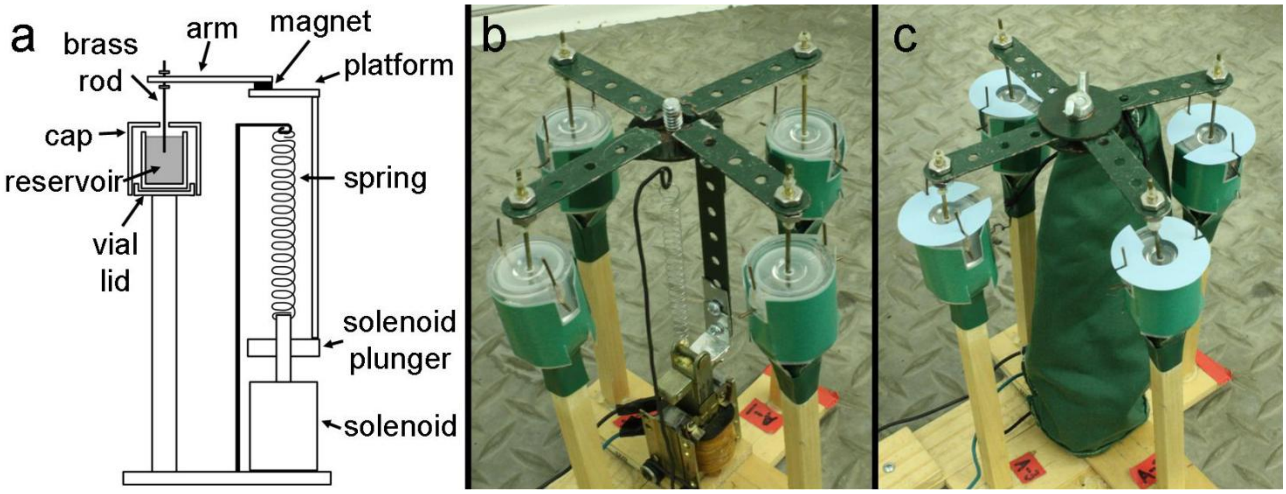

Figure 1. Flobots, shown as a diagram of the major components (a), partly disassembled (b), and fully assembled (c).

and, when power to the solenoid is turned off, lifted back up by a spring. I provide a list of the parts and materials I used in the online supplementary material (Appendix I).

The reservoir is a 5 mL, 2.2 cm-diameter vial covered by a 2.8 cm-diameter plastic cap, which has a 2.4 mm-diameter hole drilled in its centre to allow the brass rod to reach the reservoir (Fig. 1a). The reservoir sits on an upturned vial lid glued to the top of a dowel (the "stem" of the flower) and is held steady by a cylinder of laminated, green paper (the "calyx") that wraps around it (Appendix II). Both the flower "stem" and the solenoid are glued to a small board forming the base of the flobot. A piece of coloured paper can be placed on top of the reservoir to serve as the flower corolla (Appendix III). (I mass-produced both calyxes and corollas using a Silhouette Cameo paper cutter.) Two L-shaped pieces of copper wire (1.0 mm diameter) are glued to the sides of the reservoir's cap to facilitate lifting the cap out of the "calyx" and to hold the "corolla" in place.

The refilling action of the flobots is powered by a linear, pull-type solenoid with a rectangular, T-shaped plunger, a stroke length of 1.6 cm, a continuous duty cycle, and a starting force of approximately 1.5N (Fig. 1a and b). The solenoid is set so that its plunger faces upwards. Heavy wire (1.5 mm diameter) holds one end of a light-weight spring (spring constant = 28 N/m) 8.5 cm above the top of the plunger (Fig. 1a and b). The other end of the spring attaches to the plunger and holds it at its highest possible position except when the solenoid is switched on, at which point the plunger is pulled down to its lowest possible position. I covered the solenoid and spring with a cloth sleeve to prevent bees from being smashed by the working parts (Fig. 1c, Appendix IV).

A platform, consisting of a 3.2 cm-diameter fender washer, is attached to the solenoid's plunger by metal brackets and mending plates (rigid strips of metal with holes spaced along their length), which hold it above the top of the spring (Fig. 1a and b). Specifically, brackets are glued, using epoxy, to the underside of the platform and to the top of the solenoid's plunger and then bolted to either end of a 11.4 cm × 1.3 cm mending plate, which holds the platform above the plunger. (The brackets I was able to obtain had only one hole available for bolting them to the mending plate, which would not have allowed them to be attached securely, so I extended each bracket by gluing a 3.8 cm × 1.3 cm mending plate to it.)

The brass rod that dips into the reservoir attaches to the platform by means of an arm, consisting of a 5.7 cm × 1.3 cm mending plate with a 1.3 cm-diameter, 3.8 mm-thick ceramic magnet glued to one end to hold it onto the platform (Fig. 1). Because the arm is held in place by a magnet, it can easily be removed from the platform for cleaning. Up to 4 arms can be attached to the platform at one time, allowing each solenoid to refill four flowers. If multiple arms are to be attached to the platform at once, I recommend super-gluing pieces of flat toothpick to the platform to prevent the magnets from touching. The arms can be held more securely to the platform if a fender washer is placed over the top of the arms and bolted tightly to the platform (Fig. 1c). I also suggest painting the platform and arms with primer and paint designed for metal surfaces to give them a more natural appearance and protect them from rust.

The brass rod that dips into the reservoir is attached to the opposite end of the arm *via* a flexible attachment allowing it to tilt easily with respect to the arm (Fig. 1). This flexible attachment has the great advantage that it allows a flobot to function well even if the end of the arm does not sit directly over the opening into the reservoir, which makes the design robust to errors in measurement or assembly. Specifically, the brass rod goes through a hole at the end of the arm and is prevented from slipping out of this hole by two hex nuts, one fixed to it above and one below the arm. The hex nuts are prevented from sliding up or down the brass rod by narrow vinyl tubing that fits snugly around the brass rod above and below each hex nut.

Flobot performance

I designed the flobots for a study of patch departure behaviour, for which I needed a large number of flowers that could be depleted and then refilled upon demand (Essenberg

& Papaj, in preparation). In that study, bees foraged one at a time on an array of 32 flowers while a researcher watched and refilled flowers when needed.

I trained bees to visit the flowers by giving the entire colony, on multiple occasions prior to beginning an experiment, access to a "training flower," which closely resembled a flobot but had a reservoir containing about 1.5 mL of sugar syrup that bees could access directly through the hole in the flower cap (Appendix V). At the beginning of each experiment, an individual *Bombus impatiens* worker that had foraged consistently throughout the previous day was presented with an array of 16 flobots in addition to the training flower (which contained only a few microliters of sugar syrup inside its reservoir and had a few droplets of sugar syrup and a dead bee on its corolla to encourage the bee to begin foraging). I repeated this process with as many bees as necessary until I had three bees that had each completed two foraging bouts on the flobots, visiting at least 10 flowers each bout.

To provide information about how many flowers might be needed, if one wished to avoid refilling flowers during a foraging bout, I recorded the number of full flowers (containing 50% sucrose) probed per foraging bout for 11 *Bombus impatiens* workers that had each previously completed 8-9 foraging bouts on the flobots.

I also measured volumes of sugar syrup provided per refill, using 1 μL and 0.2 μL microcapillary tubes, for 15 randomly-selected flowers when filled with 50% sucrose solution and when filled with 20% sucrose solution.

RESULTS AND DISCUSSION

Bees readily learned to forage from the flobots. Out of 26 bees that were allowed to forage alone on the flobots, 22 visited at least some flowers and 16 completed two bouts, visiting at least 10 flowers per bout. Most of the sugar solution presented by a flobot is in the hole that the brass rod passes through to reach the reservoir, and bees can obtain this reward using relatively natural behavior, similar to probing the area around the base of a flower's style. (For a video of a bee visiting flobots, see Appendix VI.) Each experienced forager probed an average of 20 full flowers before returning to the colony (range = 9 to 29 flowers). Sugar syrup volumes provided by the flowers were variable and were much greater when flowers were filled with 50% sucrose solution than when they were filled with 20% sucrose solution (volume of 50% solution: 2.0 ± 1.3 μL, SD; volume of 20% solution: 0.7 ± 0.6 μL, SD). It took 20-30 minutes for one person to set up an array of 32 flowers, about 5 minutes to dismantle the flobots after an experiment, and about 10 minutes to rinse all of the components after soaking them in a bleach solution.

In nature, bees typically visit much larger numbers of flowers per foraging trip than a researcher can easily provide in the laboratory. As a result, laboratory experiments often require bees to revisit the same flowers many times per foraging bout. Using flobots, researchers can provide bees with a large number of flowers, allowing more natural foraging behaviour. Although the variation in reward delivered per flower will be a short-coming for some research questions, large variation in rewards available per flower is typical in nature (e.g., Zimmerman 1988; Thakar et al. 2003; Keasar et al. 2008). Flobots, therefore, could strengthen research related to a wide variety of questions by allowing researchers to more closely mimic conditions in the field than is usually possible in the laboratory. They would be particularly useful for studies of the effects of patch configurations on trap-lining, patch departure decisions, and other aspects of foraging movements, in which requiring bees to make many revisits during a foraging bout is especially undesirable.

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APPENDICES

Additional information may be found in the online version of this article:

Appendix I. Materials List

Appendix II. Dimensions of flower "calyx"

Appendix III. Dimensions of flower "corolla"

Appendix IV. Directions for making cloth solenoid cover

Appendix V. "Training flower"

Appendix VI. Video of a single bee foraging on flobots

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