

— Note on Methodology —

ARTIFICIAL POLLEN DISPENSING FLOWERS AND FEEDERS FOR BEE BEHAVIOUR EXPERIMENTS

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Abstract—The study of foraging behaviour in plant-pollinator mutualisms has benefitted from the use of artificial flowers to manipulate floral display traits and the delivery of floral rewards. The two most common floral rewards are pollen and nectar; some pollinators, such as bees, are obliged to collect both for survival and reproduction. While flexible designs for artificial flowers providing nectar rewards abound, useful designs for artificial flowers that dispense pollen are few. This disparity mirrors a heavy emphasis on nectar collection in the study of pollinator foraging behaviour. In this study we describe a novel, easily constructed and modifiable artificial flower that dispenses flexible amounts of pollen via an ‘anther’ composed of a chenille stem. Using controlled lab assays, we show that more pulverized honeybee pollen is collected by bumblebee (*Bombus impatiens*) workers at chenille stem feeders than at dish-type feeders. We suggest that the paucity of studies examining pollinator cognition in the context of pollen rewards might be partly remedied if researchers had access to inexpensive and easily adjustable pollen-offering surrogate flowers.

Keywords: artificial flowers, behavioural assay, bumblebee, pollen collection, learning, pollinator behaviour

INTRODUCTION

Studies of plant-pollinator mutualisms make frequent and fruitful use of artificial flowers (cf. Thomson et al. 2012). With artificial surrogates researchers can precisely control and manipulate the cues that animals experience as they forage, such as floral colour, pattern, polarization, iridescence, scent, texture, and even electrical field (Kevan & Lane 1985; Giger & Srinivasan 1995; Gumbert 2000; Biesmeijer et al. 2005; Whitney et al. 2009; Clarke et al. 2013; Foster et al. 2014). Likewise, properties of the floral reward, such as quantity and quality, may also be strictly controlled in surrogate flowers (Essenberg 2015). As a consequence, researchers have employed artificial flowers to investigate how floral properties influence pollinator foraging behaviour and how that interaction might affect the fitness of both parties. For instance, simulated flowers are used to great effect in studies of pollinator cognition, learning, preference, and movement rules (e.g., Chittka & Thomson 1997; Gegeer 2005; Ohashi & Thomson 2009; Muth et al. 2015).

Almost the entirety of research on pollinator cognition and foraging behaviour concerns nectar rewards (as has been noted for some time: Plowright & Laverty 1984). Yet flowering plant species offer many kinds of floral rewards to pollinators, including resins, oils, oviposition sites, nectar,

and pollen (Simpson & Neff 1981). Of these, nectar and pollen are by far the most common (Simpson & Neff 1981; Kevan & Baker 1983). For bees and many other insects pollen is their primary source of protein; in fact, bees cannot survive without pollen in their diet as juveniles (cf. Kevan & Baker 1983; Nicolson & van Wyk 2011). Thus it is surprising that while there exist many designs for artificial flowers that dispense nectar rewards (rev. Essenberg 2015), there are comparatively few designs that dispense pollen rewards. We suggest that at least part of the overwhelming focus on nectar collection is due to a lack of tractable pollen-rewarding surrogate flowers.

What features must a good pollen-dispensing artificial flower possess? Artificial flowers should first and foremost assist the study of ecologically relevant patterns under controlled conditions. For instance, the experimenter should be able to modify features of the surrogate, such as the orientation, size, or colour of the anther and corolla, to approximate features of live flowers. Crucially, the amount of pollen that the surrogate anther dispenses should be controllable by the researcher and mimic what real flowers produce (typically very small amounts; an average of 3.55 ± 0.91 mg pollen: see Appendix I). Furthermore, because surrogate flowers are often deployed in large arrays and researchers may need to replace flowers quickly during or between experiments (such as when bees scent mark flowers, deplete rewards or when the experimenter desires to change the distribution of rewards; e.g. Gegeer & Laverty 2005; Biernaskie & Gegeer 2007; Witjes and Eltz 2009), an ideal surrogate is inexpensive and easily assembled.

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TABLE I: Summary of artificial anthers capable of presenting pollen

Surrogate anther design	How pollen presented	Pollen (mg) per flower used	Reference
Dish	Upward-facing, in heap	500; 250-5,000; 3,000	Vaissière & Vinson 1994; Cook et al. 2003; Nicholls & de Ibarra 2014
Feather	Horizontally, on chick feathers	~20	Konzmann & Lunau 2014
Fur	On artificial fur	Undetermined [†]	Plowright et al. 1999
Fibrous*	On fibers*	Undetermined*	Thomson et al. 2012
Pin	Upward-facing, via electrostatic attraction	4,475 grains**	Stone & Thomson 1994
Syringe	Downward facing, within syringe***	500	Konzmann & Lunau 2014
Nototribic	Falls through slit on contact	Undetermined*	Thomson et al. 2015

*Dispenses food dyes, but authors state the design could be modified to dispense pollen. As such, it is unclear whether pollen adheres to the surrogate anthers / falls through the slit and whether bees will collect from them.

[†]Dispenses an unknown quantity of pulverized honeybee-collected pollen.

**Dried *Narcissus* pollen; total wet weight of similarly sized grains is calc. 0.195 – 0.236 mg (Brown & Irving 1973)

***Honeybee-collected pollen that was washed first.

The corollas of existing surrogate flower designs for pollen collection (reviewed in Tab. I) are often easy to construct and could conceivably be modified to accommodate many kinds of floral display traits. In contrast, the surrogate anthers that present the pollen reward in these designs are less modifiable (see Tab. I). For instance, dish-type surrogate anthers can only present their pollen in a heap on an upward-facing surface. Additionally, whereas nectar volumes in some surrogates resemble those in nature, pollen in most existing designs are presented in unnaturally large amounts to facilitate collection by bees (A. Russell pers. obs.) and to prevent the wing action of bees from blowing all the pollen away as they land (Intermill 1960; e.g. Appendix VI).

The design of the surrogate flower is not the only challenge that researchers face in studying pollen foraging behaviour. To encourage bees to forage for pollen outside of the nest-box the colony should be provided with an external pollen source (A. Russell pers. obs.; F. Muth pers. comm.). To our knowledge, researchers have used either of two types of feeders to train bees to forage for pollen: dish-type feeders (Kitaoka & Nieh 2009; Jandt et al. 2009; Arenas & Farina 2012; Hagbery & Nieh 2012; Cao 2014) or syringe-type feeders (Konzmann & Lunau 2014). Both designs have limitations. The pollen in dish-type feeders quickly clumps and can gradually become soaked with bee frass (see Fig. 1), which eventually renders the pollen unattractive or difficult to collect. In syringe-type feeders, if honeybee-collected pollen is used, it must first be washed to prevent clumping due to the stickiness of the nectar (A. Russell pers. observation). Washing is not only laborious, but more importantly, the process significantly alters the natural properties of the pollen. Pollen ruptures when hydrated (Engel et al. 1997; D'Amato et al. 2007), thereby exposing bees to compounds (e.g. pollen-internal proteins and starches; Roulston & Cane 2000) they likely do not experience while foraging (it may, however, be possible to wash pollen in a buffer solution to reduce osmotic rupture).

In addition, washing results in at least a partial loss of the pollenkitt (Engel et al. 1997; Teppner 2008), thereby altering pollen's adhesive qualities and phagostimulatory effects (Dobson & Bergström 2000; Lin et al. 2013).

To overcome these limitations, we describe here an inexpensive and readily modifiable artificial surrogate anther that allows bees to forage for biologically realistic amounts of free pollen, limited only by the precision of the experimenter's measurements. Additionally, we present several possible surrogate anther-flower arrangements that mimic live flower configurations. Finally, we describe the design of a novel pollen feeder and test it against dish-type feeders.

MATERIALS AND METHODS

Artificial flower design

The critical component of our artificial pollen flowers is a chenille stem (i.e. pipe cleaner) that presents the pollen reward. Other aspects of the artificial flower can be manipulated freely. We therefore present 5 simple configurations as examples, in which a 1 - 3 cm length piece of a chenille stem was hot-glued to construction paper (Fig. 2). See Appendix II for video of pollen collection behaviour on an artificial flower with 1 mg of free *Prunus avium* pollen.

Chenille stems hold pollen loosely, but relatively securely. With access to a microbalance it is therefore possible to load precise amounts of pollen (for examples see Muth et al. 2015, 2016; Francis et al. 2016). With the caveat that some pollen may be blown away by wing action, it is practical to load anthers with pollen and weigh them before and after a visit to determine pollen removal.

Chenille stem feeder design

We constructed each feeder from a 40 dram styrene vial measuring 49 (outer diameter) × 85 mm (BioQuip

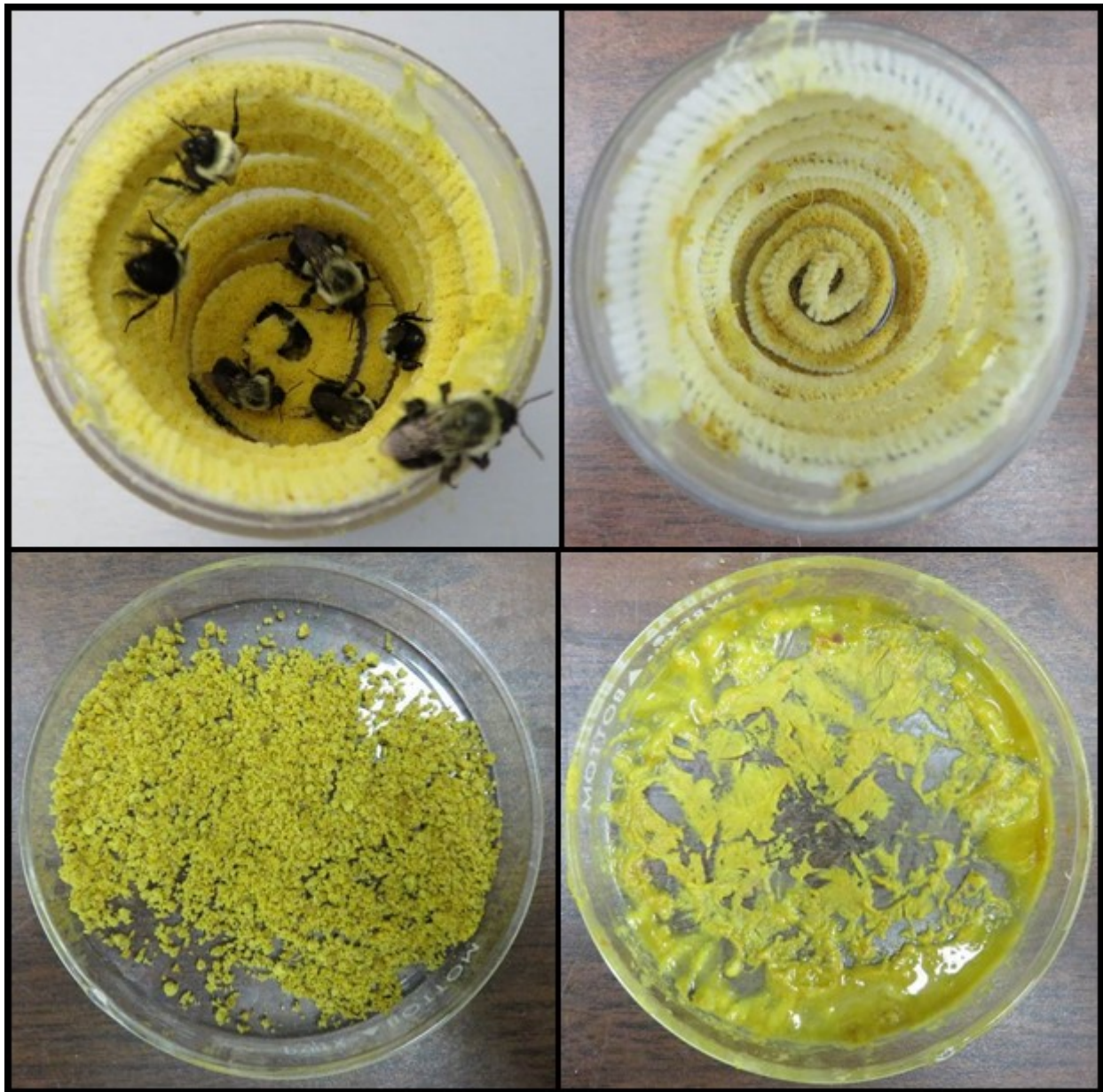


FIGURE 1. Chenille stem (top panels) and dish (bottom panels) feeders shortly after they are loaded with 4 grams of pulverized honeybee pollen (right panels) and 24 hours after being foraged in (left panels).

Products, Inc., USA), a polystyrene 53 mm, 3 oz. vial measuring 52 mm (inner diameter) \times 70 mm (Parkway Plastics, Inc., USA) (Fig. 3A), and 5 white chenille stems measuring 12 in \times 6 mm (Jo-Ann Stores, LLC).

To construct feeders, the bottom of the 40 dram vial was first sawed off (Fig. 3B). Four chenille stems were coiled tightly around the outside of the vial (Fig. 3C) and then hotglued within the 40 dram vial at regular intervals in a corkscrew pattern (Fig. 3D). We maintained a separation of approximately 0.5 cm between coils. The last chenille stem was coiled tightly and placed within the bottom of the 3 oz. vial (Fig. 3E). We coiled the chenille stems to maximize the surface area on which pollen could be presented. To form the base of the feeder, the 40 dram vial was placed within the 3 oz. vial, such that the two fit snugly together (Fig. 3F). This design element made the feeder easier to clean, as the

40 dram vial could be removed and hot water run through it. To load the feeder, honeybee-collected pollen (Koppert Biological Systems, Howell, MI, USA) was pulverized within a coffee grinder and placed within the feeder. With the cap in place, the feeder was shaken vigorously side to side and any excess pollen dumped out. The same technique is used with free pollen, except without use of a coffee grinder.

Experiments testing the chenille stem feeder

In the following experiments we sought to compare the efficacy of the feeder with a petri dish-type feeder. We chose this comparison in particular because dish feeders are by far the most common pollen feeder type used to date in the literature.

We used a minimum of two and a maximum of three colonies in experiments, purchased from Koppert Biological

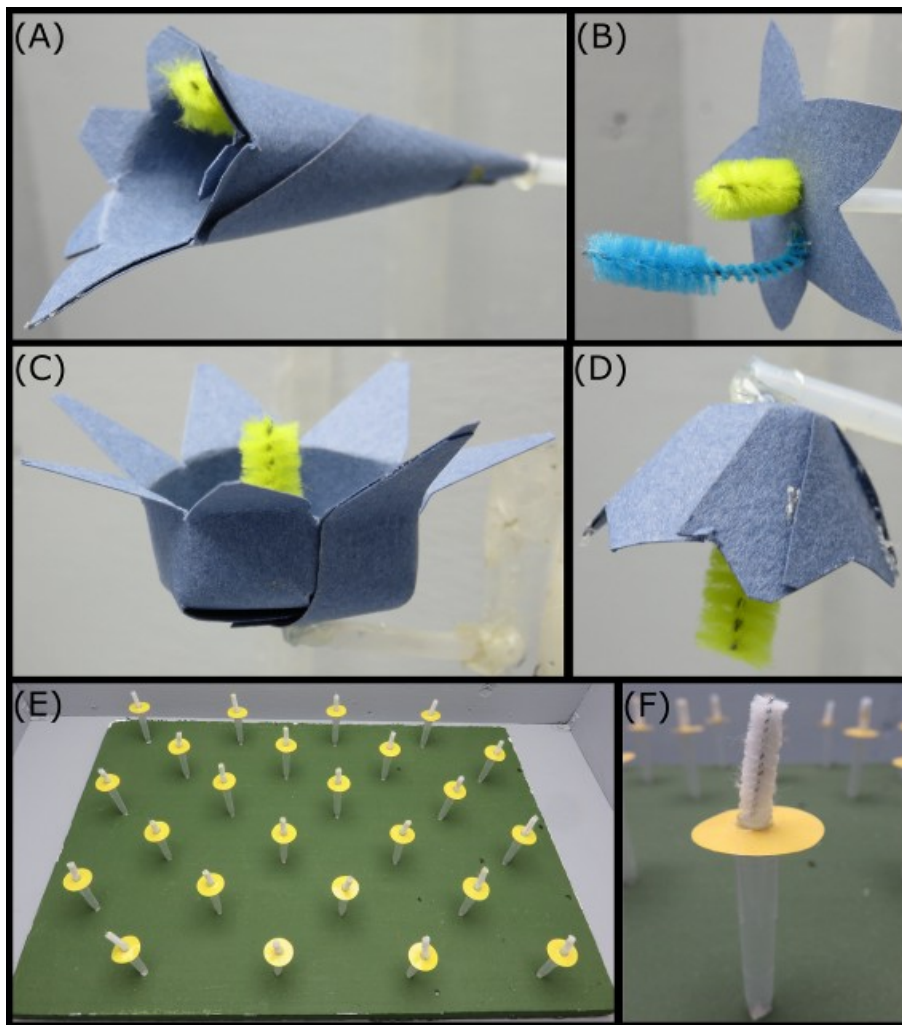


FIGURE 2. Various artificial flower configurations utilizing our surrogate anther: designs mimicking (A) trumpet flowers, (B) heterantherous flowers (with a semi-cryptic anther), (C) upward-facing flowers, and (D) pendant flowers. (E) An array composed of (F), an upward-facing flower composed of an unornamented corolla and anther.

Systems (Howell, MI, USA). We allowed bees to forage daily for sucrose solution and pollen in either of two foraging arenas ($L \times W \times H = 82 \times 60 \times 60$ cm and $82 \times 60 \times 30$ cm). The arenas had clear acrylic ceilings and were lit from above by 40 W and 60 Hz fluorescent lights (Lithonia Lighting).

On days where colonies were not being tested, colonies had access to *ad libitum* 2M sucrose solution and pulverized honeybee-collected pollen (Koppert Biological Systems, MI, USA) within the foraging arena. Sucrose solution was dispensed via braided cotton wicks (6 inch Braided Cotton Rolls, Richmond Dental) that extended into 40 dram vials (BioQuip Products, Inc., USA). Pollen was presented within chenille stem feeders and within dish feeders (glass petri dishes, 9 cm diameter), where it was spread out uniformly on the bottom of the feeder, and within chenille stem feeders.

On testing days, colonies had access to *ad libitum* 2M sucrose solution from two feeders and 4 g of pulverized honeybee-collected pollen presented in a single feeder. Each week that colonies were being tested pollen was presented via chenille stem feeders on one day and via dish feeders on the other day. We systematically alternated which feeder was used on the first day of testing for each week. Immediately prior to testing, bees in the foraging arenas were returned to the colonies and the arenas cleaned with water and with

70 % ethanol. Testing on each day began at approximately 9:30 am.

Experiment 1: Foraging activity and weight of pollen loads by feeder type

Here we sought to determine if foraging effort (number of pollen foraging bees and weight of pollen loads of returning foragers) varied by feeder type. We used three colonies.

We tested three colonies on two consecutive days during each of five consecutive weeks. Starting three days prior to each weekly test all bees that were in the foraging arena (presumed to be foraging for pollen and/or nectar) from two colonies were labelled with individually numbered plastic coloured tags (The Bee Works, Ontario, Canada) attached by superglue to the dorsum of the thorax and returned to the colony box. To identify these bees we collected any bees found in the foraging arena at 30 minute intervals for four hours per day, for two consecutive days.

Each day of testing ended after five consecutive hours of observations. Every 30 minutes the numbers of bees with and without visible pollen loads at the pollen feeder were counted. Each day of testing thus resulted in 8-10 scan samples of foraging activity per colony.



FIGURE 3. Chenille stem feeder design: (A) the 3 oz and 40 dram vials, (B) the bottom of the 40 dram vial sawed off, (C) the chenille stems coiled, (D) the coiled stems glued within the feeder, (E) coiled stem placed at the bottom of the 3 oz vial, (F) 40 dram vial placed into the 3 oz vial with pollen and cap, ready to shake.

All workers returning from the foraging chamber with visible pollen loads (identified as any bee with any amount of pollen attached to the corbiculae) were trapped via plastic removable gates set into the clear plastic tunnel that connected the foraging arena to the nest box. These bees were allowed to exit through a section of tubing that connected to the tunnel, into which collection vials could be attached.

Once collected, we moved bees into a Queen Marking Tool (The London Bee Company Ltd, Middlesex, UK), removed the complete pollen load from their right leg using fine forceps, and returned the bee to the colony box. We removed pollen only from a single leg to avoid reducing a bee's motivation to collect pollen on future bouts (A. Russell pers. obs.). Pollen loads were weighed to the nearest milligram using a Sartorius Analytic Balance (Data Weighing Systems, Inc.). Of 62 pollen foragers, we included for analysis only those bees that foraged from both feeder types over the course of the experiment (a total of 18 bees). Each bee made an average of three foraging trips to each feeder type and we used the average pollen load weight for analyses.

Experiment 2: Foraging duration by feeder type

Here we sought to determine if the duration of foraging trips varied by feeder type. We used two colonies.

A week prior to the start of this experiment all bees that were in the foraging arena from two colonies were labelled

with individually numbered plastic coloured tags as described above.

We tested colonies on two consecutive days during each of two consecutive weeks, starting the week after Experiment I had been completed. Pollen feeders were monitored continuously for 3 hours on each testing day. We noted the times when labelled bees first entered the feeder and when they left the foraging arena. Of 54 pollen foragers identified, we included for analysis 24 bees that foraged from both feeder types over the course of the experiment (13 bees from one colony, 11 from the other). Each bee made an average of three foraging trips to each feeder type and we used the average trip duration for analyses.

Data Analyses

All data were analysed using R v.3.2.0 (R Development Core Team).

We analyzed whether the duration of foraging trips, weight of pollen loads, or number of bees in a feeder per scan sample differed with the feeder type. If assumptions of normality and equal variance were met (using Shapiro-Wilk and F tests, respectively, in the mgcv package: Wood 2015), we ran paired t-tests or, otherwise, Wilcoxon-signed rank tests to analyse differences.

To analyse the effect of treatment and time of day on the number of bees in a feeder we used linear mixed-effects

models (LMERs), specifying type II Wald chisquare (χ^2) tests through the `Anova()` function in the `car` package (Fox 2015). For these models we included 'ColonyID' as a random factor and the fixed effects 'scan sample' (scan samples 1-10 per day) and 'treatment' ('chenille stem feeder' and 'dish feeder'). LMERs were carried out using the `lmer()` function in the `lmerTest` package (Kuznetsova et al. 2015).

For all LMERs, maximal models were run first. For each analysis, we performed two rounds of backward elimination (as described in Fox 2015). We checked first whether any interaction terms should be eliminated from the model and then whether any main effects should be removed. We used the `Anova()` function in R to examine significance for each of these effects relative to the full model.

RESULTS

Description of pollen foraging behaviour at feeders

Bees foraged from dish feeders by pressing their body into the loose pollen while spread-eagled, shaking their abdomen and legs from side to side while moving forward. When bees did not groom pollen into their corbiculae in flight, they lifted their back end and hind legs off the surface of the dish and groomed the pollen from their thorax and abdomen into their corbiculae.

Bees foraging from chenille stem feeders nibbled on the stem with their mandibles and scrabbled it with their forelegs. This behaviour appeared to loosen pollen, which fell onto their thorax and abdomen. When bees did not groom pollen into their corbiculae in flight, they lifted their back end and hind legs off the chenille fiber and groomed the pollen from their thoraxes and abdomen into their corbiculae. This behaviour differed conspicuously from collection behaviour seen in dish feeders and appeared more similar to collection behaviour seen on live flowers with unconcealed pollen (see Appendix III for video of bee collecting pollen from *Begonia descoleana*).

Pollen foraging activity differed by feeder type

During scan samples, significantly more bees at the chenille stem feeder were found to have a visible pollen load than at the dish feeder (Fig. 4B; Wilcoxon rank sum tests, chenille stem versus dish: $W = 1783$, $P < 0.0001$, $N = 152$ scan samples).

This result appears to be partly due to significantly more bees being found at the chenille stem feeder (Fig. 4A; Wilcoxon rank sum tests, chenille stem versus dish: $W = 1848.5$, $P < 0.0002$) and partly due to a significantly greater proportion of the bees at the chenille stem feeder having pollen loads (Fig. 4C; Wilcoxon rank sum tests, chenille stem versus dish: $W = 1511$, $P < 0.022$).

The pollen load mass of returning foragers did not vary with feeder type

We found no significant difference in the mean weight of the pollen loads of foragers returning from chenille stem feeders compared to those returning from dish feeders (Mean load mass in mg \pm SE, Chenille stem: 5.9 ± 0.07 ,

Dish: 6.8 ± 0.07 ; paired t -test, chenille stem versus dish: $t_{17} = 0.9082$, $P = 0.3765$, $N = 18$ bees).

Foraging duration did not vary with feeder type

We found no significant difference in the mean duration of foraging trips for bees collecting pollen at chenille stem feeders compared to those collecting at dish feeders (Mean trip duration in minutes \pm SE, Chenille stem: 21.7 ± 1.77 , Dish: 19.1 ± 1.36 ; Wilcoxon rank sum tests, chenille stem versus dish: $V = 189$, $P = 0.2712$, $N = 24$ bees).

Diel trend in foraging effort did not vary by feeder type

We found a non-significant tendency for the number of bees at a feeder to decline over the course of a day and no significant interaction between temporal pattern and feeder type (Type II Wald χ^2 tests: bees in feeder versus scan sample: $\chi^2 = 2.2132$, $df = 1$, $P = 0.1368$; bees in feeder versus feeder type: $\chi^2 = 46.5609$, $df = 1$, $P < 0.0001$; bees in feeder versus scan sample:feeder type: $\chi^2 = 0.0963$, $df = 1$, $P = 0.7564$; $N = 76$ scan samples).

We likewise found a non-significant tendency for the number of bees with visible pollen loads to decline over the course of a day and no significant interaction between temporal pattern and feeder type (Type II Wald χ^2 tests: bees in feeder versus scan sample: $\chi^2 = 2.4764$, $df = 1$, $P = 0.1156$; bees in feeder versus feeder type: $\chi^2 = 60.2173$, $df = 1$, $P < 0.0001$; bees in feeder versus scan sample:feeder type: $\chi^2 = 0.0506$, $df = 1$, $P = 0.8220$; $N = 76$ scan samples).

Although foraging effort did not decline over the course of each daily observation period and did not vary by feeder type, by the morning of the following day we observed no bees foraging within the dish feeder, although there were still bees collecting pollen from the chenille stem feeder.

DISCUSSION

The advantages of using chenille stems as surrogate anthers are numerous. Pipe cleaners are inexpensive, obtainable at many arts & crafts stores, available in a variety of colours (or can be dyed), and are easily trimmed to adjust the length of the chenille stem (and fibers, although not with precision). Bumblebees readily forage from artificial flowers possessing these surrogate anthers and already three publications to date have relied on our design to study pollen foraging behavior (e.g. Muth et al. 2015, 2016; Francis et al. 2016). While our results seem likely to be extended readily to other species of bumblebees, it is worth exploring the value of the artificial anther and the feeder for those species, as well as honeybees and other bees. Moreover, the artificial anther may prove useful in presenting pollen to other taxa known to forage for pollen, such as flies and beetles (cf. Kevan & Baker 1983).

Our chenille stem feeder is easy to assemble and repair. If the researcher washes the feeder's elements frequently to prevent the sugars in honeybee pollen from growing mold, the feeder can be reused for months and even years (our own feeders are yet to wear out after over a year of continuous

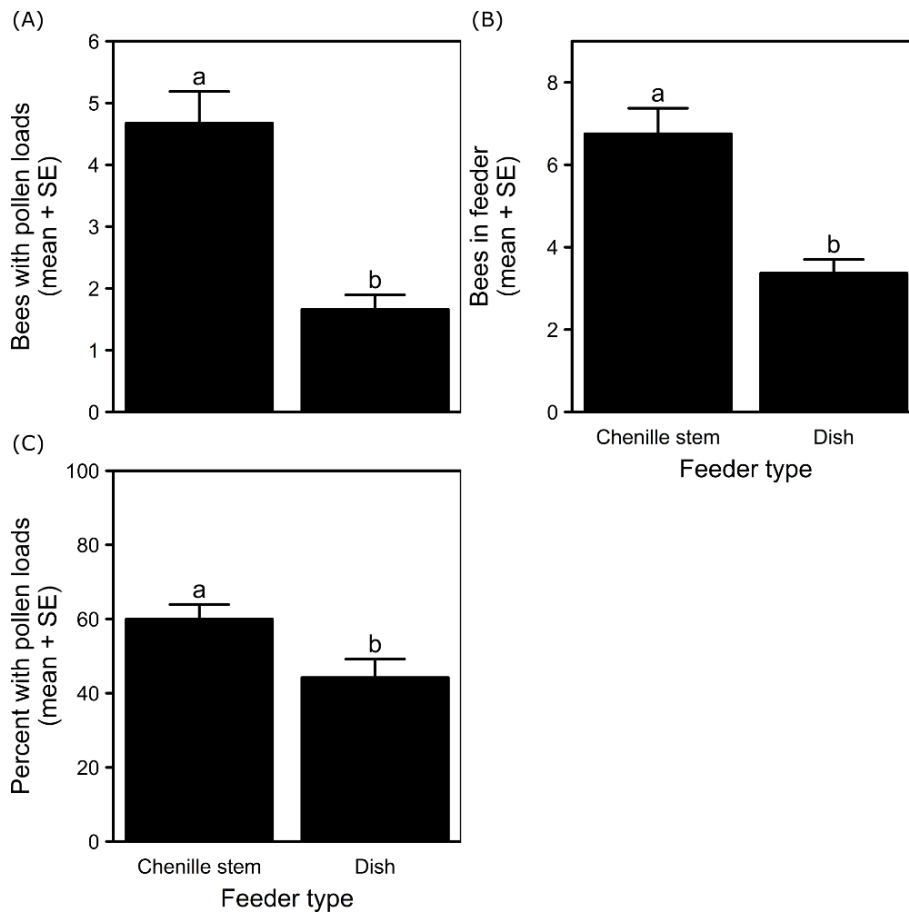


FIGURE 4. (A) Mean number of bees within the chenille stem and dish feeders that had visible pollen loads. (B) Mean number of bees within the chenille stem and dish feeders. (C) Mean percentage of bees within the chenille stem and dish feeders that had visible pollen loads. $N = 76$ scan samples for chenille stem and dish feeders. $N = 3$ colonies per treatment. Letters above bars within a panel indicate significant differences at $P < 0.05$ according to a Wilcoxon rank sum test.

use). With established colonies we recommend that pollen in chenille stem feeders be replenished daily. Although anecdotal observations on over 20 colonies suggest that young colonies will continue successfully foraging from these feeders over two days, we recommend switching out the pollen more frequently to reduce the buildup of scent marks and mold.

In addition to describing a novel feeder, we demonstrated the efficacy of our chenille stem feeder over the standard petri dish feeder, a design that has been used for over 20 years. Bees are able to collect pollen as quickly and as fully from either type of feeder. However, bees are less likely to collect from the dish feeder. Specifically, three times as many bees were observed collecting pollen from chenille stem feeders as from dish feeders. We speculate that this difference may be due to bees being arrested more on the chenille stem feeder, perhaps due to bees perceiving the pollen-covered stem as more similar to a real anther as they grasp it. As a result of greatly reduced pollen collection, colonies fed from petri dish feeders might produce smaller and fewer workers than they otherwise would. Because we tested our feeder designs using honeybee-collected pollen, our results are relevant for any study that relies on bee-collected pollen presented in petri dishes to feed bees. However, although we used bee-collected pollen in our feeders, the chenille stem design works well with free pollen (A. Russell pers. obs.) and we have no reason to believe that the results reported here would not be replicated with the use of free pollen.

We used honeybee-collected pollen in our feeders for three reasons. First, bee-collected pollen is very cheap (\$15 USD per 2.2 kg) compared to commercially available entomophilic free pollen (\$1 - \$5 USD per gram). While it is certainly possible for a researcher to collect pollen manually, collecting even a few grams is generally extremely labor intensive. Second, bee-collected pollen is available year-round in virtually unlimited quantities, whereas the availability of free pollen is dependent on crop flowering season and demand by farmers. Third, bee-collected pollen is a mix of pollen from numerous floral species, which is probably a more realistic larval diet for polylectic bumblebees than the comparatively taxonomically-poor commercially available free pollens. For all of these reasons, we anticipate that researchers will feed bee-collected pollen to bumblebee colonies for the foreseeable future, hence our decision to use such pollen in our experiments.

While the use of honeybee-collected pollen for colony maintenance is probably unavoidable at present, we recommend the phasing out of bee-collected pollen as a reward in pollen foraging experiments intended to understand natural processes. Even though honeybee-collected pollen has been frequently used in the study of pollen foraging behavior (e.g. Kitaoka & Nieh 2009; Arenas & Farina 2012; Hagbery & Nieh 2012), it is a poor match to the properties of fresh floral pollen. First, this pollen is adulterated with up to 60% regurgitated crop sugars (e.g. Davis 1997; Human & Nicolson 2006), non-pollen debris (even thrips: e.g. Davis 1997), and probably digestive fluids

and cuticular honeybee waxes. Second, bee-collected pollen is a mix of pollen from numerous floral species, many of which may not be readily collected by bumblebees (Leonhardt & Bluthgen 2012). While no study has directly assessed differences in using bee-collected pollen versus free pollen on foraging behavior, it is easy to think of several. For instance, any learned association between floral cues and a honeybee-collected pollen reward might be due at least in part to its nectar content.

To study foraging behavior under ecologically realistic conditions, researchers are accustomed to providing bees with per-flower nectar volumes that approximate what real flowers offer (e.g. Gruter et al. 2011). The same reasoning applies to the study of pollen foraging. For this reason we reviewed the literature to provide a thorough accounting of the weight of pollen offered by individual flowers of 157 species, across 103 genera and 44 families (Appendix I). Additionally, knowledge of the amount of nectar rewards offered by flowers has helped us to understand how pollinators should forage optimally for nectar (cf. Essenberg et al. 2015). We hope our data summary will likewise assist in the creation of a complementary literature for pollen rewards and for when pollinators, such as bees, should forage for both floral resources.

A variety of pollen-based studies could be facilitated by our surrogate anther. For instance, a standardized artificial anther could be used to study how bees gather pollen, by allowing a fine-scale assessment of motor routines as they vary among bee taxa or in relation to bee size. Or, for example, researchers could more easily investigate the role of pollen and anther visual and olfactory cues by dyeing chenille stems to visually conceal pollen rewards. Many broad questions related to pollinator cognition have scarcely been addressed with respect to pollen rewards or their combination with nectar rewards. Floral constancy, multimodal learning, or pollinator movement rules might also benefit from a surrogate anther that can be modified to present a variety of floral cues and quantities of pollen, to name just a few potential research areas.

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APPENDICES

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

Appendix I. Mean weight of pollen offered per flower for various entomophilous species

Appendix II. Bumblebee foraging for free *Prunus avium* pollen on a chenille stem

Appendix III. Bumblebee foraging for unconcealed pollen from *Begonia descoleana*

Appendix IV. Bumblebee foraging for free *Prunus avium* pollen within a small petri dish

REFERENCES

- Antoń S, Denisow B, Milaniuk K (2014) Flowering, pollen production and insect visitation in two *Aconitum* species (Ranunculaceae). *Acta Agrobotanica* 67: 3-12.
- Arenas A, Farina WM (2012) Learned olfactory cues affect pollen-foraging preferences in honeybees, *Apis mellifera*. *Animal Behaviour* 83: 1023-1033.
- Atlin G (1984) Farmer maintenance of TPS varieties. In: *Innovative Methods for Propagating Potatoes: Report of the XXVIII Planning Conference, December 10-14, 1984, Lima, Peru: International Potato Center*, pp 38-62.
- Biernaskie JM, Gegear RJ (2007) Habitat assessment ability of bumble-bees implies frequency-dependent selection on floral rewards and display size. *Proceedings of the Royal Society B* 274: 2595-2601.
- Biesmeijer JC, Giurfa M, Koedam D, Potts SG, Joel DM, Dafni A (2005) Convergent evolution: floral guides, stingless bee nest entrances, and insectivorous pitchers. *Naturwissenschaften* 92: 444-450.
- Bosch J, Retana J, Cerdá X (1997) Flowering phenology, floral traits and pollinator composition in a herbaceous Mediterranean plant community. *Oecologia*, 109: 583-591.
- Cao N (2014) Life histories and energetics of bumble bee (*Bombus impatiens*) colonies and workers. Doctoral dissertation. Retrieved from UA Campus Repository. [online] URL: <http://hdl.handle.net/10150/323419> (accessed December 2015)
- Chittka L, Thomson JD (1997) Sensori-motor learning and its relevance for task specialization in bumble bees. *Behavioral Ecology and Sociobiology* 41: 385-398.
- Clarke D, Whitney H, Sutton G, Robert D (2013) Detection and learning of floral electric fields by bumblebees. *Science* 340: 66-69.
- D'Amato G, Liccardi G, Frenguelli G (2007) Thunderstorm-asthma and pollen allergy. *Allergy* 62: 11-16.
- Davis AR (1997) Further miscellaneous constituents of corbicular pollen loads from *Apis mellifera*: petals, stamens, anther threads, and thrips. *Acta Horticulturae* 437: 199-206.
- DeGrandi-Hoffman G, Thorp R (1991) The influence of nectar and pollen availability and blossom density on the attractiveness of almond cultivars to honeybees. *Acta Horticulturae* 288: 299-302.
- Denisow B (2008) Flowers ecology and pollen output of *Symphytum officinale* L. *Journal of Apicultural Science*, 52: 81-89.
- Denisow B, Zuraw B (2002) Blooming biology and pollen presentation of flowers from genus *Helleborus* L. *Sectio EEE, Horticultura* 10: 45-50.
- Denisow B, Antoń S (2012) Flowering, nectar secretion, pollen shed and insect foraging on *Aquilegia vulgaris* L. (Ranunculaceae). *Acta Agrobotanica* 65: 36-44.
- Denisow B, Antoń S, Szymczak G (2013) The flowering, pollen production, and insect visitors in the ornamental shrub *Potentilla fruticosa* L. (Rosaceae). *Journal of Apicultural Science* 57: 95-105.
- Dobson HEM, Bergström G (2000) The ecology and evolution of pollen odors. *Plant Systematics and Evolution* 222: 63-87.
- Engel E, Richter K, Obermeyer G, Briza P, Kungl AJ, Simon B, Auer M, Ebner C, Rheinberger H-J, Breitenbach M, Ferreira F

- (1997) Immunological and biological properties of Bet v 4, a novel birch pollen allergen with two EF-hand calcium-binding domains. *The Journal of Biological Chemistry* 272: 28630-28637.
- Essenberg C (2015) Flobots: Robotic flowers for bee behaviour experiments. *Journal of Pollination Ecology* 15: 1-5.
- Essenberg CJ, Easter RA, Simmons RA, Papaj DR (2015) The value of information in floral cues: bumblebee learning of floral size cues. *Behavioral Ecology*. doi: 10.1093/beheco/arv061
- Fleming TH, Sahley CT, Holland JN, Nason JD, Hamrick JL (2001) Sonoran desert columnar cacti and the evolution of generalized pollination systems. *Ecological Monographs* 71: 511-530.
- Foster JJ, Sharkey CR, Gaworska VA, Roberts NW, Whitney HM, Partridge JC (2014) Bumblebees learn polarization patterns. *Current Biology* 24: 1415-1420.
- Fox J (2015) *Applied regression analysis and generalized linear models*, 3rd ed. USA: Sage Publications, Inc.
- Francis J, Muth F, Papaj DR, Leonard AS (2016) Nutritional complexity and the structure of bee foraging bouts. *Behavioral Ecology*. Accepted.
- Gavilánez-Slone JM (2000) Pollination and pollinators of pumpkin and squash (*Cucurbita maxima* Duchesne) grown for seed production in the Willamette valley of western Oregon. Master's thesis. Retrieved from ScholarsArchive@OSU. URL: <http://hdl.handle.net/1957/32908>
- Gegeer RJ. (2005). Multicomponent floral signals elicit selective foraging in bumblebees. *Naturwissenschaften*. 92: 269-271.
- Gegeer RJ, Laverty TM (2005) Flower constancy in bumblebees: a test of the trait variability hypothesis. *Animal Behaviour* 69: 939-949.
- Giger AD, Srinivasan MV (1995) Pattern recognition in honeybees: eidetic imagery and orientation discrimination. *Journal of Comparative Physiology A* 176: 791-795
- Gonzales VM, Coque M, Herrero M (1994) Pollinator selection in kiwifruit (*Actinida deliciosa*). *Journal of Horticultural Science* 69: 697-702.
- Grüter C, Moore H, Firmin N, Helanterä H, Ratnieks FLW (2011) Flower constancy in honey bee workers (*Apis mellifera*) depends on ecologically realistic rewards. *The Journal of Experimental Biology* 214: 1397-1402.
- Gumbert A. (2000). Color choices by bumble bees (*Bombus terrestris*): innate preferences and generalization after learning. *Behavioral Ecology and Sociobiology* 48: 36-43.
- Hagberg J, Nieh JC (2012) Individual lifetime pollen and nectar foraging preferences in bumble bees. *Naturwissenschaften* 99: 821-832.
- Hill SJ (1985) Almond pollination studies: pollen production and viability, flower emergence and cross-pollination tests. *Australian Journal of Experimental Agriculture* 25: 697-204.
- Intermill RW (1961) Pollen preferences and factors which influence pollen collection by the honey bee *Apis mellifera* L. Master's degree. Retrieved from Kansas State University Libraries. URL: <https://archive.org/details/pollenpreference00inte>
- Jandt JM, Huang E, Dornhaus A (2009) Weak specialization of workers inside a bumble bee (*Bombus impatiens*) nest. *Behavioral Ecology and Sociobiology* 63: 1829-1836.
- Kevan PG, Baker HG (1983) Insects as flower visitors and pollinators. *Annual Review of Entomology*. 28: 407-453.
- Kevan PG, Lane MA (1985) Flower petal microtexture is a tactile cue for bees. *Proceedings of the National Academy of Sciences* 82: 4750-4752.
- Kitaoka TK, Nieh JC (2009) Bumble bee pollen foraging regulation: role of pollen quality, storage levels, and odor. *Behavioral Ecology and Sociobiology* 63: 500-510
- Kołtowski Z (2001) Beekeeping value and pollination requirements of double-improved cultivars of spring rapeseed (*Brassica napus* L. var. *oleifera* Metzger f. *annua* Thell. *Journal of Apicultural Science* 45: 69-84.
- Kuznetsova A, Brockhoff PB, Christensen RHB (2015) Tests in Linear Mixed Effects Models. R package version 2.0-29. [online] URL <https://cran.r-project.org/web/packages/lmerTest/index.html> (accessed December 2015)
- Labarca C, Loewus F (1972) The nutritional role of pistil exudate in pollen tube wall formation in *Lilium longiflorum*. *Plant Physiology* 50: 7-14.
- Leonhardt SD, Blüthgen N (2012) The same, but different: pollen foraging in honeybee and bumblebee colonies. *Apidologie* 43: 449-464.
- Lin H, Gomez I, Meredith JC (2013) Pollenkitt wetting mechanism enables species-specific tunable pollen adhesion. *American Chemical Society* 29: 3012-3023.
- Masierowska M, Piętko T (2014) Variability in nectar and pollen production in flowers of double-low lines of white mustard (*Sinapis alba* L.) and their attractiveness to honey bees. *ACTA Scientiarum Polonorum* 13: (197-209.
- Muth M, Papaj DR, Leonard AS (2015) Bees remember flowers for more than one reason: pollen mediates associative learning. *Animal Behaviour* 111: 93-100.
- Muth M, Papaj DR, Leonard AS (2016) Colour learning when foraging for nectar and pollen: bees learn two colours at once. *Biology Letters* 11: 20150628. URL <http://dx.doi.org/10.1098/rsbl.2015.0628>
- Nicholls E, Hempel de Ibarra N (2014) Bees associate colour cues with differences in pollen rewards. *Journal of Experimental Biology* 217: 2783-2788.
- Nicolson SW, van Wyk JH (2011) Bee food: the chemistry and nutritional value of nectar, pollen and mixtures of the two. *African Zoology* 46: (197-204.
- Ohashi K, Thomson JD (2009) Trapline foraging by pollinators: its ontogeny, economics and possible consequences for plants. *Annals of Botany* 103: 1365-1378.
- Passarelli L, Cocucci A. (2006) Dynamics of pollen release in relation to anther-wall structure among species of *Solanum* (Solanaceae). *Australian Journal of Botany* 54: 1-7.
- Percival M (1950) Pollen presentation and pollen collection. *New Phytologist* 49: 40-63. doi:10.1111/j.1469-8137.1950.tb05143.x
- Percival M (1955) The presentation of pollen in certain angiosperms and its collection by *Apis mellifera*. *New Phytologist* 54: 353-368. doi:10.1111/j.1469-8137.1955.tb06192.x
- Plowright CMS, Cohen-Salmon D, Landry F, Simonds V (1999) Foraging for nectar and pollen on thistle flowers (*Cirsium vulgare*) and artificial flowers: how bumble bees (*Bombus impatiens*) respond to colony requirements. *Behaviour* 136: 951-963.
- Plowright RC, Laverty TM (1984) The ecology and sociobiology of bumble bees. *Annual Review of Entomology*, 29: 175-199.
- Poulton JL, Bryla D, Koide RT, Stephenson AG (2002) Mycorrhizal infection and high soil phosphorus improve vegetative growth and the female and male functions in tomato. *New Phytologist* 154: 255-264.

- R Development Core Team (2010) R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing.
- Roulston TH, Cane JH (2000) Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution* 222: 187-209.
- Schmidt JO, Buchmann SL (1986) Floral biology of the saguaro (*Cereus giganteus*). *Oecologia* 69: 491-498.
- Simpson BB, Neff JL (1981) Floral rewards: alternatives to pollen and nectar. *Annals of the Missouri Botanical Garden* 68: 301-322.
- Snow AA, Roubik DW (1987) Pollen deposition and removal by bees visiting two tree species in Panamá. *Biotropica*, 19: 57-63.
- Stawiarz E, Wróblewska A (2014) Flowering of Siberian peashrub (*Caragana arborescens* Lam.) and its forage value to pollinating insects. *Acta Agrobotanica*, 3:13-20.
- Stawiarz E, Wróblewska A (2013) Flowering dynamics and pollen production of *Laburnum anagyroides* Med. under the conditions of south-eastern Poland. *Journal of Apicultural Science*, 57. <http://dx.doi.org/10.2478/jas-2013-0021>
- Szklanowska K, Dabska B (1993) Sugars and pollen yield of some trees and shrubs from Fabaceae family. *Annales Universitatis Mariae Curie - Skłodowska, sectio EEE, Horticultura* 1: 103-106.
- Teppner H (2008) The easiest proof for the presence of pollenkitt. *PHYTON Annales Rei Botanicae* 48: 169-328.
- Thomson JD, Draguleasa MA, Tan MG (2015) Flowers with caffeinated nectar receive more pollination. *Arthropod-Plant Interactions* 9:1-7. DOI 10.1007/s11829-014-9350-z
- Thomson JD, Ogilvie JE, Makino TT, Arisz A, Raju S, Rojas-Luengas V, Tan MGR (2012) Estimating pollination success with novel artificial flowers: effects of nectar concentration. *Journal of Pollination Ecology* 9: 108-114.
- Turner V (1984) Banksia pollen as a source of protein in the diet of two Australian marsupials *Cercartetus nanus* and *Tarsipes rostratus*. *Oikos* 43: 53-61.
- Van Tets, IG (1996) Pollen in the diet of Australian mammals. Doctoral dissertation. Retrieved from University of Wollongong Thesis Collection. URL: <http://ro.uow.edu.au/theses/1051>
- Whitney HM, Kollé M, Andrew P, Chittka L, Steiner U, Glover BJ (2009) Floral iridescence, produced by diffractive optics, acts as a cue for animal pollinators. *Science* 323: 130-133.
- Witjes S, Eltz T (2009) Hydrocarbon footprints as a record of bumblebee flower visitation. *Journal of Chemical Ecology* 35: 1320-1325.
- Wood S (2015) Mixed GAM computation vehicle with GCV/AIC/REML smoothness estimation. R package version 1.9-9. [online] URL: <https://stat.ethz.ch/R-manual/R-devel/library/mgcv/html/mgcv-package.html> (accessed December 2015)
- Wróblewska A, Stawiarz E (2012) Flowering abundance and pollen productivity of *Ligularia clivorum* Maxim. and *Ligularia przewalskii* Maxim. *ACTA Scientiarum Polonorum* 11: 57-67.
- Yeaman RL, Roulston TH, Carr DE (2014) Pollen quality for pollinators tracks pollen quality for plants in *Mimulus guttatus* *Ecosphere* 5: 1-8.
- Żuraw B, Denisow B (2002) Biologia kwitnienia i pylenie kwiatów z rodzaju *Helleborus* L. *Annales Universitatis Mariae Curie-Skłodowska* 10: 45-50.
- Żuraw B, Sulborska A, Stawiarz E, Weryszko-Chmielewska E (2015) Flowering biology and pollen production of four species of the genus *Rosa* L. *Acta Agrobotanica* 68: 267-278.