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A 3D PRINTED POLLEN TRAP FOR BUMBLE BEE (BOMBUS) HIVE ENTRANCES

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Corresponding Author:	James P. Strange, PhD Ohio State University Columbus, Ohio UNITED STATES
Corresponding Author's Institution:	Ohio State University
Corresponding Author E-Mail:	strange.54@osu.edu
Order of Authors:	Houston J. Judd
	Craig Huntzinger
	Ricardo Ramirez
	James P. Strange, PhD
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TITLE:
A 3D Printed Pollen Trap for Bumble Bee (*Bombus*) Hive Entrances

AUTHORS AND AFFILIATIONS:
Houston J. Judd [1,2], Craig Huntzinger[2], Ricardo Ramirez[1], James P. Strange [2,3]

[1]Department of Biology, Utah State University, Logan, UT, USA
[2]USDA-ARS Pollinating Insect Research Unit, Logan, UT, USA
[3]Department of Entomology, The Ohio State University, Columbus, OH, USA

Corresponding Author:
James P. Strange
Email Address: strange.54@osu.edu
Tel: (614) 292-9325

Email Address of Co-Authors:
Houston Judd (houstonjudd@gmail.com)
Craig Huntzinger (craig.huntzinger@ars.usda.gov)
Ricardo Ramirez (ricardo.ramirez@usu.edu)

KEYWORDS:
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SUMMARY:
We present a non-lethal and automated mechanism to collect pollen from bumble bee (*Bombus*) workers returning to a hive. Instructions for producing, preparing, installing and using the devices are included. By using 3D-printed objects, modification to the design was timely, efficient and allowed for quick turnaround for testing.

ABSTRACT:
To verify the plant sources from which bumble bees forage for pollen, individuals must be collected to remove their corbicular pollen loads for analysis. This has traditionally been done by netting foragers at nest entrances or on flowers, chilling the bees on ice, and then removing the pollen loads from the corbiculae with forceps or a brush. This method is time and labor intensive, may alter normal foraging behavior, and can result in stinging incidents for the worker performing the task. Pollen traps, such as those used on honey bee hives, collect pollen by dislodging corbicular pollen loads from the legs of workers as they pass through screens at the nest entrance. Traps can remove a large quantity of pollen from returning forager bees with minimal labor, yet to date no such trap is available for use with bumble bee colonies. Workers within a bumble bee colony can vary in size making size selection of entrances difficult to adapt this mechanism to commercially reared bumble bee hives. Using 3D printing design programs, we created a pollen trap that successfully removes the corbicular pollen loads from the legs of returning bumble bee foragers. This method significantly reduces the amount of time required by researchers to collect pollen from bumble bee foragers returning to the colony. We present

the design, results of pollen removal efficiency tests, and suggest areas of modifications for investigators to adapt traps to a variety of bumble bee species or nest box designs.

INTRODUCTION:

Bumble bees (*Bombus* spp.) are large robust insects that are found across the temperate, alpine, and arctic regions of the world[1]. They are important to plant communities and provide important pollination service for the agricultural crops that they visit[2]. Recent declines in the abundance and distribution of several species has brought their importance as pollinators to the forefront of public awareness[3]. Researchers have identified several stressors that are likely contributing to population declines including a lack of diverse and abundant floral resources on which bumble bees forage[4]. Identifying which plant species bumble bees forage from allows researchers and land managers to understand how bumble bees may be responding to changes in resource availability, competition, and anthropogenic disturbances[5,6].

Studies investigating the pollen foraging preferences of bumble bees are often conducted by researchers catching individual bees foraging at flowers, and then removing the corbicular pollen loads from specimens for further processing and identification[7-10]. While this method provides insight into how a species or an assemblage of bumble bee species utilizes the resources in an area[7], it is time intensive and potential differences in preferences among hives cannot be discerned without additional molecular analyses to identify colony of origin of the foraging bee[11]. For some studies of foraging dynamics, it is desired to conduct the studies at individual colonies; however, wild bumble bee nests are generally located underground or at ground level making them difficult to locate[12]. Commercially produced bumble bee hives provide researchers greater access and better experimental control and the removal of pollen off workers is still primarily conducted by capturing foragers as they return to the hive and manually removing their corbicular pollen loads[13, 14]. The removal of pollen by hand from the corbicula of a bee is time intensive with a low hourly yield of pollen especially at hive entrances where the rate of returning pollen foragers may be low. Additionally, manually removing pollen from bees can result in stings from disturbed workers.

Pollen traps have been used for experimental removal of pollen from honey bees for decades[15]; yet, a passive method for removing pollen from bumble bees has not been developed. The primary obstacle in developing a mechanism to remove pollen from returning forager bumble bees is the large variation of worker sizes that exist in a bumble bee colony[16]. Honey bee pollen traps are effective largely because honey bee worker size does not vary much. Additionally, these traps require only minor manipulations after installation and don’t require bees to be sacrificed[17]. This is achieved using screens or plastic surfaces that dislodge the pollen off of the hind legs of workers as they return to the hive. These traps remove only a portion of the pollen loads from returning foragers and the various designs of those result in varied efficiencies at pollen collection. As the pollen is removed from the bee legs, it falls through a screen and into a collection basin to which the bees have no access, so that the researcher can remove it with only minor disturbance to the hive.

The purpose of the present study is to adapt the techniques used for collecting pollen from honey bee hives and apply them to bumble bee nests using 3D printed structures and test the trap designs on colonies of *Bombus huntii*. The design process followed the assumptions that the traps should be inexpensive to produce, adaptable to a variety of bumble bee species, cause minimal harm or disturbance to the bees, and that the rate of pollen removal should exceed hand collection of pollen. Three-dimensional printing technology is versatile, easily accessible, and a cost-effective tool allowing researchers to replicate and modify objects for specific purposes[18]. The technique presented here instructs the user to build pollen traps and attach them onto commercially available bumble bee colonies. The traps are not designed to be use with wild colonies. These traps passively remove the corbicular pollen loads from the hind legs of pollen carrying bumble bees as they return to their nest boxes.

PROTOCOL:

1. Print pollen trap structures

1.1. Download the appropriate STL file for the nest box that bumble bees are nesting in (e.g., Biobest or Koppert style hives, https://www.ars.usda.gov/pacific-west-area/logan-ut/pollinating-insect-biology-management-systematics-research/docs/pollen-traps/). The files are available to the public, free for download and modification by the end user.

1.2. Open the STL file in the printer program. Follow printer manufacturer directions to build the four trap components.

NOTE: Allow approximately 3 h for the trap body to print, 2 h for catch basin to print and 30 min each for the filter and trap closure insert to print. Trap body size is 6 cm x 3.8 cm x 7 cm.

2. Pollen trap assembly

2.1. Remove the support structures printed with the trap body and catch basin including those of the sieve structure of the trap body (**Figure 1**).

2.2. Use a 3/16 in. (0.476 cm) drill bit mounted in a hand drill to clear any plastic strands crossing the raised edges of the pollen filter that might hinder a bee from moving through the filter holes. Use a box cutting razor blade and sandpaper to even out any bumps or raised edges on the flat side of the pollen filter.

2.3. Place the pollen filter in the trap body by gently pushing the plastic filter through one side of the trap body. The filter will only fit in one way, as the left side of the trap has a larger opening to accommodate passage of the raised filter cones.

2.3.1. If the side slits are too small for the pollen filter to slide through smoothly, scrape enough plastic away from the slit in the trap body with a razor or other tool that can remove small portions of plastic at a time. Ensure that the pollen filter fits securely in place with no more than a 2 mm gap between the pollen filter and trap body.

2.4. Attach the catch basin to the trap body by sliding the raised edges on the bottom of the trap body into the groove on the top of the catch basin. The catch basin should be positioned directly underneath the sieve region of the trap body (**Figure 1A–E**).

2.5. Make appropriate modifications by cutting or sanding the plastic to allow smooth placement and removal of the catch basin from the trap body. Placement of the catch basin will secure the pollen filter into place and it cannot be removed until the catch basin is removed, nor can the pollen filter be inserted while the catch basin is in place.

NOTE: If multiple hives are placed close to one another, providing each hive with a unique color combination of the trap body, catch basin and pollen filter structures in addition to deploying hives with varied orientation will help returning workers find their nests.

3. Bumble bee colony preparation

3.1. Stop pollen feeding 24–48 h before deploying colonies. This will cause workers to use up any stored pollen and stimulate them to leave the nest in search of pollen.

3.2 Prepare the hive trap body for installation by inserting a trap closure insert into the filter slot to prevent bees from escaping while installing the trap.

3.3 Working under red light to prevent the bees from flying, lift the plastic nest box out of the cardboard outer box.

3.4 Locate the plastic nest entrance at the front of the nest. There are two styles of entrance depending on the nest supplier: Koppert-style boxes (step 3.5) and Biobest-style boxes (step 3.6).

3.5. For Koppert-style hive entrances, mount the pollen trap onto the nest entrance by pulling up on the entrance tab until both entrance holes are open.

3.5.1. Insert the two tubes of the pollen trap into the entrance holes, ensuring that the sieve of the pollen trap is on the bottom. Gently push down on the plastic entrance tab to secure the pollen trap in place.

3.6. To install the pollen trap into Biobest-style hive entrances, use a flat head screwdriver to gently pry the plastic entrance device from the nest box. Insert the pollen trap into the nest entrance holes until the pollen trap is firmly against the nest (**Figure 1E**).

3.6.1. Secure the trap to the nest using tape or quick dry glue where the trap contacts the nest box if needed.

3.7. Return the plastic nest box to the cardboard box. Cardboard may need to be cut away to accommodate the pollen trap.

4. Deployment of nests

4.1. Place nest boxes into the study area. Provide cover to protect from precipitation and anchorage for wind as these can adversely affect the quality and quantity of pollen that is collected. Provide hives placed in greenhouses with adequate sun cover to reduce overheating.

4.2. Remove the trap closure insert from the trap body to allow bees to forage freely so that foragers can orient themselves with the surrounding area and the location of their nest. Orientation flight time should be complete in 24 h under normal conditions.

5. Pollen collection

5.1. To engage the trap, slide the pollen filter (pollen filter) into the filter slot, ensuring that it is securely in place.

5.2. Install the catch basin by sliding it onto the trap body from the front until it is fully closed. If the catch basin is excessively loose or falls off the trap body, use a rubber band to secure it to the trap body.

5.3. Observe bees entering and exiting the pollen trap at first deployment to ensure the pollen filter holes are large enough to accommodate the bees.

5.3.1. If workers are unable to pass through the pollen filter, remove the filter and then use drill bits larger than 3/16 in. (0.476 cm) to increase the holes sizes. Do so in a sequential manner, increasing hole diameter 1/32 in. (0.079 cm) each time, as holes that are too large will not collect any pollen.

5.3.2. Once bees are able to pass through the filter, continue to observe the entrance to ensure pollen is being removed upon re-entry.

NOTE: Bees should have some difficulty moving though the pollen filter holes, especially the first few times they pass through. If they pass through too easily, the pollen may not be dislodged from the corbiculae.

5.4. After the designated period of pollen collection, slide and remove catch basin from the trap body.

5.5. Process the pollen loads according to your experimental design.

5.6 Remove the pollen filter to allow workers to forage freely until the next period of pollen collection. The trap body may remain attached to the hive for the duration of the experiment.

NOTE: Pollen traps may be engaged for as long as the researcher desires to collect pollen from a colony. However, deployment of pollen traps for over 24 h in a week may result in starvation of brood in a hive and retard colony development.

REPRESENTATIVE RESULTS:

Eight different pollen filter designs were tested to determine their efficacy and efficiency at removing corbicular pollen loads from returning bumble bee workers. All designs were successful at removing at least of one corbicular pollen load from a returning forager. However, some were found to slow workers from leaving or entering the hive or failed to remove pollen loads (**Table 1).** Pollen traps with various filters were tested sequentially on 4 laboratory reared colonies of *B. huntii* Greene foraging on *Phacelia tanacetifolia* grown in greenhouses for a cumulative total of 138.5 h and 229 corbicular pollen loads (**Table 1**) collected over the 7-day period (3/2/16–3/8/16). Video cameras were placed in front of the nest entrances while pollen traps were engaged to record forager activity. Trap entrance design proceeded by trial and error over that period. Fifty-two hours of video observation and 142 corbicular pollen loads were collected during the test period (**Table 1**). Efficiencies were calculated by dividing the number of corbicular pollen loads collected by the number of observed pollen laden foragers that passed through a filter. Pollen filter design efficiencies ranged from 2–58.9% of full corbicular pollen loads removed. Corbicular pollen loads were removed from the legs and fell as cohesive pellets of pollen into the catch basin. Because of this tendency for corbicular loads to be removed as a pellet, partial corbicular pollen load removal was uncommon, but some partial loads may have been counted as full removal because we could not verify that some pollen remained in a corbicula after the bee entered the nest. Overall filter openings that were circular improved pollen collection and movement of workers into the nest environment. In addition, filter designs that had raised structures that extended away from the nest box also improved pollen removal from the hind legs of foragers. In a previous field study using an earlier filter design, the average weight of the pollen that was collected following 24 h of collection was 1.017 g over 11 hive-day collection periods. There was high variation (0.22–2.94 g per day) among the total mass of pollen collected from each nest. These values represent an expected mass range that pollen may be collected using this method. The final design in the download pollen trap print file is design number 8, a circular trap entrance with raised edges.

FIGURE AND TABLE LEGENDS:

Figure 1: Pollen trap mounted to bumble bee hive. (**A**) Front view of pollen trap where workers land and travel across the sieve towards the pollen filter. (**B**) posterior view of assembled pollen trap showing the grooved edges of the trap body that allow the catch basin to slide on and attach. (**C**) Side view of assembled pollen trap showing the pollen filter slit which allows the pollen filter to be placed into the trap body and secured by the catch basin. (**D**) Bottom view of trap body with pollen filter inserted, the sieve enables corbicular pollen loads to fall into the catch basin and restricts workers from accessing collected pollen. (**E**) Side view of assembled pollen trap attached to a nest box.

Figure 2: Mechanism by which corbicular pollen loads are removed from legs of workers. (**A**) side view of a worker and its relative size to an assembled pollen trap. (**B**) Side view of worker approaching a pollen filter hole. (**C**) Side view of worker passing through a pollen filter hole forcing the corbicular pollen loads to contact the filter and ventral surface of the abdomen. (**D**) Posterior view of worker passing through a pollen filter hole forcing corbicular pollen loads to contact the filter and ventral surface of the abdomen. (**E**) Posterior view of worker passing through pollen filter hole once corbicular pollen loads have been stripped from its corbiculae and drop through the sieve and into the catch basin, and (**F**) side view of worker passing through pollen filter hole once corbicular pollen loads have been stripped from its corbiculae and drop through the sieve and into the catch basin.

Table 1: Summary table of the total deployment hours, corbicular pollen loads collected, collection rate along with the hours, number of pollen foragers, individual and total efficiency verified through video footage.

DISCUSSION:

Collection of pollen from bumble bee colony entrances can allow for a variety of ecological and agricultural studies. Identifying the floral sources from which bumble bees collect pollen provides valuable information and insight into the diversity of plants that contribute to a colony's overall diet[19]. Identifying the pollen source has implications for both agricultural production and studies of ecosystem services in wild lands[12, 20]. By gathering relatively large samples sizes of corbicular pollen loads, researchers can determine if bumble bees are foraging on the target crop for which they were deployed[21], other important constituents of the bumble bee diet[8], and preferred forage of specific species within an area[7]. Automating pollen collection from bumble bee colonies by using a pollen trap will allow for expanded studies of bumble bee foraging, nutrition and pesticide exposure.

Hand removal of pollen from bees, which has been used for the majority of studies investigating bumble bee pollen preferences, is time and labor intensive[10]. In contrast, passive collection of pollen using the pollen trap design collected over 200 corbicular pollen loads from four colonies over the course of a one-week period. Thus, this method allows researchers to collect pollen from multiple hives across different locations increasing both sampling effort and statistical robustness for future studies.

To develop and improve the pollen filter designs, video recording of the bumble bee workers passing through the trap mechanism was essential. We observed that when a bumble bee passes through a tight space, the hind legs extend behind and underneath its abdomen (**Figure 2C–E**). Observation of this behavior resulted in changes to the 3D printing design and trial and error testing of pollen filters (**Table 1**). Utilization of the video recordings for observation when deploying traps is recommended prior to initiation of the formal experiment to ensure that the traps are functioning properly and efficiently so that modifications can be made if necessary. To account for differences in body size both within a hive and among hives and the goals of the study, trap entrance size can be varied. We observed that adjusting the trap entrance to allow the larger foragers to exit and enter the hive provided minimal disruption to foraging activities and reasonable efficiency (>50%) removal of corbicular pollen loads. The 3D-printed plastic is easily modified with hand tools and print designs can be manipulated for specific projects or as box entrance designs change[18].

The limitations of this method are that pollen filter designs are unique to the species of bumble bee that is being sampled. This method uses a uniform size of entrance holes which, in theory, may restrict the larger individuals in the nest from foraging and smaller workers that forage may not be sampled; however, our design permitted large workers to pass through and we did not quantify efficiency based on body size. The design available for download is designed on the average size of *B. huntii* workers (3.22 mm thoracic width[22]) and thus that would need to be expanded as described for workers of *B. impatiens* (3.38 mm[23]) or *B. terrestris* (4.77 mm[24]). In one study using *B. terrestris*, larger individuals were noted to collect pollen more often than smaller workers[25]; however, a subsequent work found no correlation in worker size and the frequency of pollen foraging trips of the North American bumble bee *B. impatiens*[26,27,28]. Additionally, worker size may vary throughout the season[12], thus pollen filters should be inspected regularly to ensure that pollen collection is occurring efficiently. Understanding the species of interest and the specific research questions being addressed will be critical in assessing the utility of this trap design on a case-by-case basis.

Behavioral responses exhibited by returning foragers to the presence of engaged pollen traps were variable. These included: (i) workers acclimating to the additional effort needed to re-enter the nest environment, (ii) workers taking multiple attempts to pass through the filter, (iii) workers attempting to circumvent the filter opening and instead to pass through the trap body sieve, and (iv) workers avoiding the filter and switching to nectar gathering and transferring to nest bees through ventilation holes along the bottom of the nest box. Bumble bee foragers attempting to find alternative entrances to the hive had been observed in a previous study of this species[29] even when nest entrances are not blocked. While these responses were observed they were uncommon enough that we did not quantify the proportion of foragers who altered behavior due to the trap presence, except that most bees acclimated to the traps soon after trap deployment. Future applications of this method include adapting existing designs for other commercially produced bumble bee species, particularly *B. terrestris* and *B. impatiens* which are primarily used for the pollination of greenhouse crops worldwide[19]. Use of these pollen traps on commercial hives in locations outside of their native range will allow researchers to determine what niche overlaps and competitive interactions may be occurring with native *Bombus* species[30,31].

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DISCLOSURES:
The authors have nothing to disclose.

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Figure

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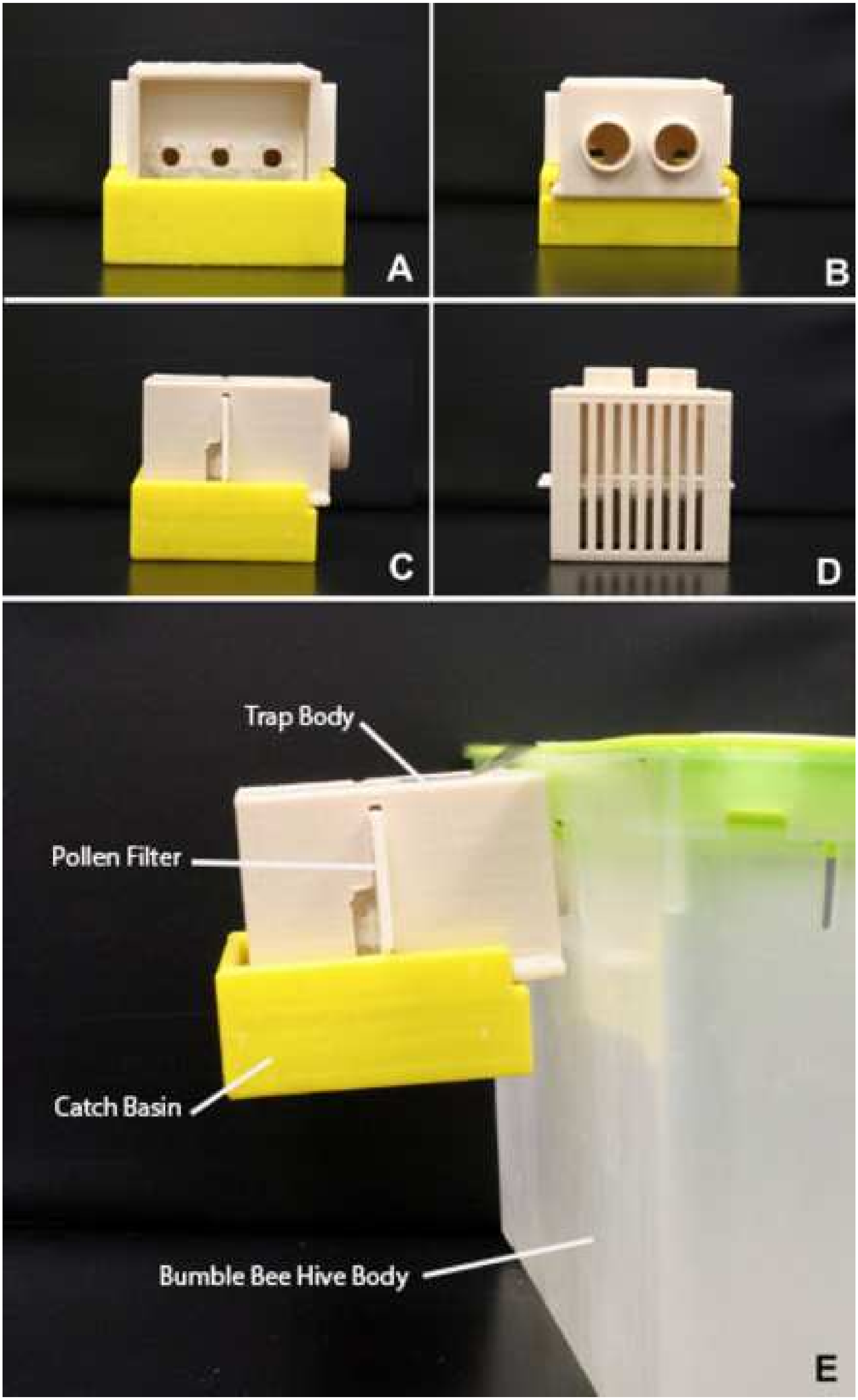

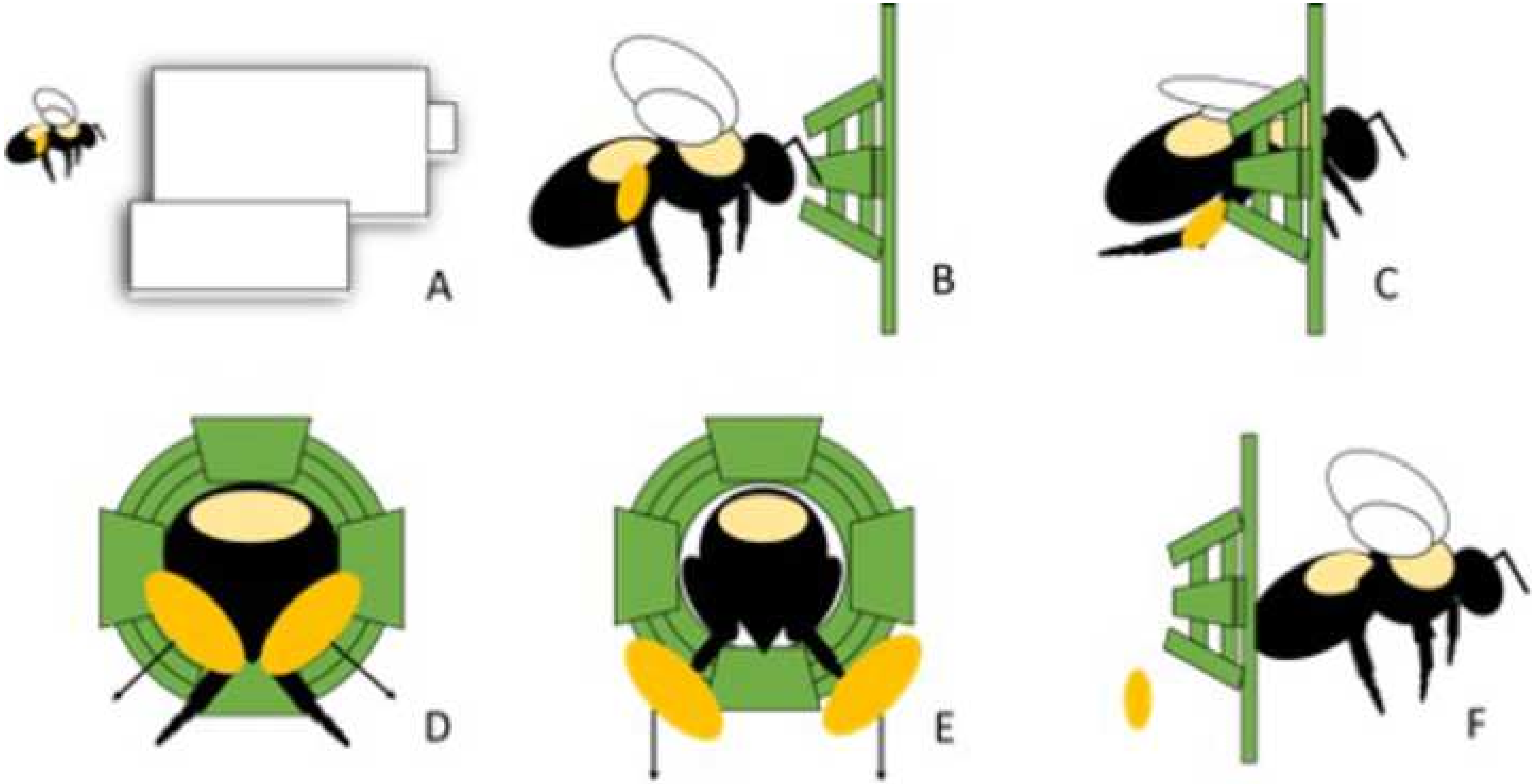
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Table 1.

		Cumulative Totals			
Design ID	**Entrance Shape**	**Deployment (Hours)**	**Corbicular Pollen Loads Collected**	**Collection Rate (Pollen Loads/hour)**	**Observation (Hours)**
1	Diamond	1	1	1	1
2	Diamond	3.5	2	0.57	3.5
3	Square	4.5	2	0.44	4.5
4	Circle	9.5	7	0.74	-
5	Circle	17.5	10	0.57	5.75
6	Circle	18	36	2	13
7	Circle	49.5	48	0.97	6.25
8	Circle	35	123	3.51	18
	Total	138.5	229	-	52

*Average number of corbicular pollen loads collected from returning pollen foragers.

**Percentage of total corbicular pollen loads collected from returning foragers (Individua

Video Observation			
Corbicular Pollen Loads Collected	Pollen Foragers	Individual Efficiency*	Total Efficiency**
1	9	0.11	5.56%
2	50	0.04	2.00%
2	2	1	50.00%
-	-	-	-
5	23	0.22	10.87%
35	54	0.65	32.41%
11	17	0.65	32.35%
86	73	1.18	58.90%
142	228	-	-

al/2).

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
MakerBot Replicator+	MakerBot	Model PABH65	
MakerBot Tough Material	PLA Filament		various colors
Nest Box	Biobest		Not sold publicly without bee purchase

JoVE61500 Judd et al. Response to reviewer comments (Response in *italics*)

Please find the attached responses to reviewer and editorial comments along with the revised manuscript.

Please note that for some reason the line numbering changed after line 149. I do not know why or how to fix it. I have googled this and attempted to revert it back to continuous numbering, but to no avail. I sincerely apologize and my comments on lines follow this new numbering.

Editorial Comments:

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors. *Done.*

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please ensure that all specific details (e.g. button clicks for software actions, numerical values for settings, etc) have been added to your protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. *Done.*

• **Protocol Numbering:** Please adjust the numbering of your protocol section to follow JoVE's instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step. *I am sorry. I have tried to adjust this, but I don't know if I got the numbering right. Other aspects have been corrected.*

• **Protocol Highlight:** Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps. *Done.*
1) Some of your shorter protocol steps can be combined so that individual steps contain 2-3 actions and maximum of 4 sentences per step.
2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.
3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.
4) Notes cannot be filmed and should be excluded from highlighting.

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol. *I have made changes to the discussion based on editorial and reviewer* *comments.*

• **Figure/Table Legends:** Add a common figure title to Fig 1. *Done.*

• **References:**
1) Remove hyperlinks from the text and list them among the references. (See lines 97-102). *Done.*
2) Avoid citing unpublished work (see line 259). *Removed.*

• **Commercial Language:** JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are MakerBot®, Onshape Inc, Autodesk, Tinkercad, etc
I removed the note on the design of the trap and it is not part of the methods presented.
1) Please use MS Word's find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by "(see table of materials)" to draw the readers' attention to specific commercial names.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]." *The figures are original, I took the photos and Houston Judd made the drawings.*

Reviewer #1:
Manuscript Summary:
The authors present the plans for a pollen trap to collect the pollen loads of bumble bee workers (B. affinis) returning to their colony, as well as a small amount of data indicating the effectiveness of the trap. I've been looking forward to reading this manuscript, since the community has been awaiting a useable pollen trap for bumble bees for years! The writing in general was clear and a pleasure to read.

Major Concerns:
While I really appreciated the attention to a detailed protocol for assembling and using the

pollen trap, I was disappointed that the authors did not take the opportunity to prove the effectiveness of the trap. For instance: is there a forager size bias in terms of whose loads are more likely to be removed? Is there a pollen size load bias in terms of what loads most likely to be removed? Are loads removed completely or only partly? How does pollen foraging rate of colonies with a pollen trap compare to colonies without a pollen trap? All of these questions and probably more would need to be answered to determine whether this pollen trap provides unbiased sampling for reasonable ecological metrics. I think that the Discussion should also address these potential limitations and strengths. Further, I think it would greatly increase the value of this study if the authors have data addressing these and related questions.

We do not have data addressing all of these issues, unfortunately. That would have constituted a much larger study and our goal was to design a pollen trap to look at both bumble bee diets and field exposure to pesticides. Two papers following up on this directly address some of these experimental questions but do not seem appropriate to a methods paper showing development and implementation of the pollen traps which is a more methodological approach. However, concerning the pollen size load bias this is addressed in the representative results where we state the percentage of loads removed. I added some clarifying text in lines 208-209.

Further, while using a non-commercially available bumble bee like Bombus huntii is definitely valuable and to be commended, nearly all laboratory and most field studies are performed with B. impatiens or B. terrestris, whose foragers have well known body size ranges and average body sizes: testing this pollen trap on a rarely used bumble bee species whose body size is not reported in this paper (it would be very nice if this comparison were made to B. impatiens and B. terrestris) means that researchers wanting to use this pollen trap with other Bombus species will have to do their own substantial trial and error. I think that the Discussion should also address these potential limitations and strengths.

*Bombus huntii is available for purchase in western Canada. It is illegal to possess Bombus terrestris in the United States and Bombus impatiens is not permitted to be used in several western states due to concerns for invasive potential of the species. In fact, Bombus impatiens is an invasive species in British Columbia and Washington (Looney et al. 2019), thus using a native western bumble bee seemed to be an ecologically responsible choice for this research project. In lines 276-277 we clearly state "*The limitations of this method are that PF designs are unique to the species of bumble bee that is being sampled.*" So I do not feel we hid this at all. It is simply a limitation of the region in which our study was conducted.*

Likewise, the authors mention on L284-L289 a variety of behavioral alterations generated by the trap. This seems to be absolutely crucial information, yet is relegated to the discussion rather than the methods and results. If data are available, they need to be presented: that would greatly increase the value of this study. If this is anecdotal, it needs to be described as such. Further questions that should be addressed include whether pollen foragers versus nectar foragers are similarly deterred by the traps, and whether different sizes of bees are similarly deterred. If the behavior of the bees is being systematically altered by the pollen traps, this could greatly affect the reliability of the data collected with the trap. Other potential issues might stem from the reduced pollen intake: do the authors have recommendations for how long the pollen traps can be maintained without starving the colony or altering the pollen and nectar foraging behavior of the colony? If they do not, I would suggest including that under future directions or for limitations.

I added a sentence lines 291-293 to state the observational nature of the be behavioral changes.

Table 1: It seems really difficult to make scientifically sound comparisons between the 8 designs presented in this table, which are confounded presumably with entrance shape (it's not clear what entrance shape refers to - it only appears once, in this Table) and with deployment time. It could be that some designs show numerically greater rates of collection simply because at first bees had to get used to the pollen traps and so their initial rates of success will be very low. Further, it seems each design was tested only once, and the results, I assume, seem to be the totals across all 4 colonies, rather than means +/- SE. I think the evidence for the superiority of one design over the others needs to be more clearly presented and discussed.

Our intention is not to present detailed data on all 8 trap designs as they were developed in a trail and error manner. Our detailed data is focused on the more promising designs and we present the plans for the most effective.

Finally, I found the results section to be too abbreviated in parts, making it confusing to me, and I think it would also benefit from additional explanation. Why are these 'representative results'?

These are "representative results" following the journal format.

Are these colonies outdoors?

I added text to clarify this.

Eight different pollen filter designs were tested, but only one type is shown: why?

I added text to clarify this.

What makes each of these designs better or worse? Table 1 shows 3 types of pollen filters, not all 8? Pictures would help explain L207-210. Are the total efficiency ranges calculated as an average across the 4 hives, or each hive, for each filter design?

Text added to clarify.

What is the information on L210-212 intended to convey? There does not seem to be an obvious point of comparison to the current data. Is the variation (L212) due to using the 8 different pollen filter designs? In my opinion this paragraph needs more work to clarify the methods used and what the results are intended to convey.

I am not sure the reviewer understood that this is a methods paper written to present the use of a pollen trap attached to a bee hive. Honey bee pollen trap research requires that researchers who work with them calibrate the traps when they use them. These traps will be similar in that they are a tool requiring calibration at the beginning of and during an experiment to insure they are working properly. We state this in the paragraph starting on line 277.

Minor Concerns:
L55-56: I know what you mean, but bumble bees aren't using the plants themselves as food: would recommend changing to "Identifying which plant species bumble bees forage from allows…"

I changed this
Personal preference, but is there any reason "use/s" cannot be substituted for utilize/s/tion throughout the text?

L63-L64: Not clear to me what is meant by "potential differences in preferences among hives cannot be discerned without additional analyses"

I added text to clarify.

L68: I'm not sure why the "however" is needed. The authors could combine these two sentences and point out that whether with commercial or wild bumble bee colonies, removal of pollen is conducted by capturing individual workers.
Corrected

L71: Since this is unpublished data, it's hard to say how this data came about, but this rate seems low. Researchers in our group for instance are able to remove a corbicular load roughly every 5 minutes for instance and I very much doubt we are the fastest. I do agree that whatever the manual rate is, it's going to be a LOT slower than multiple bees having their loads removed automatically as they walk through this system - so I'm not sure the specific number the authors discuss is needed. They could just point out the logic of the superiority of an automatic vs manual removal and leave it at that.

I removed the statement of rate.
L79: honey bee body size does vary, just not nearly as much as bumble bees. I would just add the word "does not vary much"
Changed as suggested
L94-L196: Are the acronyms for the various parts of the trap really necessary? It would be easier to read in my opinion if the authors referred to the parts of the pollen trap in full

each time.
done throughout
L200: "at least one". Also, do the authors mean from a given returning forager (in other words, at least 1 of 2 possible loads)?
I am not sure how to change this "one orbicular load" is the load of one corbicula.
L217-219: What is individual and total efficiency? The former term is mentioned only twice, neither with a definition. Is it 'verified' through video footage? Rather, isn't it calculated from video footage and what's in the pollen trap?
Defined in lines 210-212.
L255-261: Again, I'm not convinced a particular example needs to be described. The authors could describe this with general logic: obviously to measure multiple cites more researchers are needed, and collection is going to depend on each individual's skill, etc, and it will undoubtedly be slower than automated, simultaneous, and continuous removal of pollen loads. I would be more interested in the authors comparing their system to the total intake of what bees actually bring in hourly/daily/weekly. This would give evidence of the accuracy and reliability of this system.
removed details of the rate of collection.
L279: The Couvillon paper cited here is inappropriate: it does not test how size relates to the frequency of pollen foraging trips. Russell et al. 2017 (Patterns of pollen and nectar foraging specialization by bumblebees over multiple timescales using RFID) and Hagbery & Nieh 2012 (Individual lifetime pollen and nectar foraging preferences in bumble bees.) and references within both do examine this.

L280: Worker size can vary over the season, but much better references are: Nhi Cao's 2014 unpublished PhD thesis and Hagbery & Nieh 2012 (Individual lifetime pollen and nectar foraging preferences in bumble bees).
references added as suggested
I seem to recall that Koppert and Biobest occasionally change the designs for their colony boxes. The authors may wish to include photos of the boxes they used for their pollen trap and the dates those boxes were purchased, to allow other researchers to replicate usage in the future.
We included photos of the Biobest boxes and the printer files are made available and are easily modified if nest designes change.

Reviewer #2:
Review for 3d printed polen trap for bumblebee hive entrances.

I found this paper generally straightforward. I did not try to actually make the trap, so I cannot comment on what is really the most important part: how well the protocol and apparatus work.

I have only one major comment. How does the issue of body size work? In the paper, you seem to suggest that if you pick a medium sized hole, it will get pollen from most foragers, but miss the smaller ones and exclude the larger ones. Is this true? Won't this have fairly significant effects on the colony's foraging dynamics to have larger foragers locked out? I don't know what a solution to this problem is, and it seems inherent in polymorphic

species like bombus. I realize that you do address this in the manuscript, but I think you should make it a little more central, because it seems to be the one real problem in what is otherwise a nice piece of equipment.
I have added text in the discussion regarding this issue.

minor comments.

Lines 34-35: also note a drawback of the current method (that is, capturing and cooling) is that it disturbs behavior, and makes it difficult to conduct behavioral studies of foraging and collect pollen from incoming foragers.
modified as suggested
Line 50: sentence beginning with "they…" is awkward. Just state directly that they are important in both natural and agricultural systems.
modified as suggested
55: plants --> plant.
modified as suggested
90: implement? Do you mean "attach"?
modified as suggested
90: can they be used on natural colonies? I assume not, but you should say this explicitly.
modified as suggested
140: altering hive orientation? Do you mean having one hive face forward, the next backwards, the next forwards, etc down a row? As it reads, it sounds like you will alter the orientation while the poor bee is out foraging, which I'm sure would not help the workers find their way home!
modified for clarity
143: do you mean "remove any supplemental pollen"? How can you remove surplus pollen from the nest without ripping it apart and either getting stung or having to remove the bees first.
modified for clarity
166: what do you mean by "following recommendations of the supplier"?
modified for clarity
200: at least one… My take on this is that pollen data from these traps are qualitatively reliable (that is, you get a sample of what's coming in), but not quantitatively (more might come off one bee than another). Is this true? I don't know if this is true for apis pollen traps as well, but you should include this information.
added text to representative results to explain this
288: point 4) is interesting- can you please expand in a little more detail.
added text and a reference

Reviewer #3:
This work introduces a newly-developed pollen trapping devise for bumble bees. I found that the devise has much potential to contribute to future pollination studies of bumblebees and one of the largest advantages of proposed method may be the accessibility to the devise: anyone can make it by a 3D printer with programs open for public. The authors honestly mention a potential weak point of this devise (possible failure in

collecting pollen loads from small foragers) and give readers a proper caution. The manuscript is well-written and descriptions are clear enough. I have no major comments.

Minor comments:
line 49 "(Bombus sp.) " should be "(Bombus spp.) ". sp. and spp. should not be Italic. *corrected*
line 120 (Figure): Please indicate figure number. *corrected*
line 135 (Figure 1a): In the figure, components are indicated with upper-case letters (e.g., A, B, C…). Please unify the way for indicating figure components (with upper or lower case). *corrected*
line 195 "hive development" should be replaced with "colony development". *corrected*
lines 209 - 210 " filter designs that had raised structures… improved pollen removal…": It is unclear for me which one has such a structure in Table 1. Or, does this sentence mention about preliminary trials? Anyway, I cannot find supporting data for this description. *corrected*
line 268 (Figure 2.1): Please correct the figure number (there is no Fig. 2.1). *corrected*
Table 1: What is the difference in the design between Design ID 1 and 2, and among ID 4 - 8? Are they simple replicates with the same trap? *Text added to address this*
Figure 1: Please indicate CB, PF, TCI and TB in photos to help identifying. *corrected*

Reviewer #4:
The authors provide details and results for a trap to safely remove pollen from bumble bees when returning to their nests. This is an important advance for research on pollen use by these insects, as it will save countless hours of tedious and risky fieldwork, waiting for bees to return to their colonies and them handling them to remove pollen. The paper is well written with appropriate background and information on how to implement this technique, and requires minimal editing.
26. …returning to a hive. *Corrected as suggested*
41. …we created a pollen trap… *Corrected as suggested*
45. Replace test with tests *Corrected as suggested*
49. No italics for sp., and it should be spp. *Corrected as suggested*
81-82. Make clear here that only a small proportion of the pollen is removed from honey bees. This can help set the reader's expectation that the goal is not 100% pollen removal. *Excellent point. Edited as suggested.*
131. Replace Insure with Ensure. Also line 180 and 185. *Corrected as suggested*
For Table 2, 1 d.p. is sufficient for the total efficiency values.
I could not see a title for the Table of Materials, and it seemed to only be showing Biobest colony information. Is there another for Koppert colonies? *We did not include this here as the traps were only tested on Biobest colonies. The trap is identical except how it attaches to the colony entrance.*

Reviewer #5:
Manuscript Summary:
The authors provide a detailed methodology and proof-of-concept data for a pollen trap for

bumble bee colonies. Researchers studying bumble bees have been challenged by pollen collection at the hive entrance. This 3D pollen trap could significantly increase pollen collection efficiency for experiments and also provide important natural history details about pollen usage throughout a colony's lifetime by installing/uninstalling the trap.

Minor Concerns:
The manuscript and methods for the most part are easy to follow to replicate what the authors did. I have some specific comments in an effort to increase the clarity of the work.

Abstract
-Line 34. I have always assumed that it is also fairly traumatic for workers to be caught, their pollen removed, and then released. It seems like this pollen trap could remove that trauma for the workers as well. *Edited as suggested*
-Line 46. The abstract mentions "novel species", but it isn't clear what the authors are referring to because at this point. They haven't even said that the traps were designed for Bombus huntii colonies. We only learn that detail down in the Results. I would either remove "novel species" or instead say in the abstract that the traps were designed for B. huntii colonies but could be modified for other species of Bombus. *Added text to clarify this.*

Introduction
-Line 55. Remove "can". Should read: "...on which bumble bees forage." *Corrected as suggested*
-Line 64. Not clear what the authors mean by "additional analyses". Too cryptic. Either they should clarify what they mean here or remove that part of the sentence. *Corrected as suggested*
-Both in the Abstract and in the Introduction, the authors make the point that collecting pollen from bumble bee colonies is challenging because of the variable size of the workers within a colony. The authors then never mention how the designed pollen traps overcome that challenge. It seems like they do so by using a flexible plastic the bees have to push through, correct? That needs to be stated explicitly either in the Introduction or the Discussion. *Corrected as suggested on line 491-517.*
-Lines 90-92. The last sentence of the Introduction was poorly worded and hard to follow. Reword for clarity. *This paragraph was rewritten.*

Protocol.
-Line 120. The figure callout is missing a number and panel letter. *Corrected as suggested*
-Line 180-186. We don't learn until the Results that the traps were placed on Bombus huntii colonies. I would include that detail here, and I would also recommend including a Table either in the main text or as a supplement listing the average worker body size (or maybe variation in worker body size - mean, min, and max for workers) for the most common commercially available bumble bee species. That will then give a researcher an idea of how much they may need to modify the trap protocol to work with their species of interest prior to tinkering to get the trap to work. *I added the text to Lines 89-90 regarding Bombus huntii. Regarding body sizes, I included general data from wild caught bumble bees that occurred in the literature on lines 522-525.*

Results
-Line 209-210. I think an image or specifically pointing to features in Figure 2 may help clarify what is meant by "raised structures extending away from the nest box" *As four other reviewers did not identify this as an issue I am choosing not to add another image.*
-Line 212. I appreciated that the authors provided variation in pollen collection amounts, but it would have been nice to be able to ascribe that variation to some factor(s). Was the variation due to the traps themselves? Worker body size variation and how well the traps worked given that variation? Colony size? Colony age? *I am not sure how to respond to this. Are they suggesting an additional collection of data? If so I cannot do that until COVID-19 restrictions are lifted by my university. I also feel that would be a significantly diffrnt study than the design of a method for pollen collection.*

Discussion
-Line 268. The figure callout says (Figure 2.1). I think it should probably be (Figure 2a)? *Corrected as suggested.*
-Line 284. Add "the" in front of "presence". *Corrected as suggested.*

Figures
-Figure 1A-D. It would be nice if those images had a ruler or scale embedded at the bottom to get a sense of size. *Again, due to COVID-19 restrictions I cannot retake the photo. I have added a note on the size of traps to the text.*