The use of 3D printing to facilitate *Drosophila* behavior research.

Fingerut, Jonathan, Julia Cautela, Haley Patrick, Amelia Brown, Matthew Nelson, and Scott McRobert. Department of Biology, Saint Joseph's University, 5600 City Ave Philadelphia, PA 19131; Corresponding Author: Jonathan Fingerut, e-mail: jfingeru@sju.edu.

Studying and quantifying *Drosophila* behavior is critical for understanding their role in ecologically important interactions. For example, knowing the food choice behavior of the agricultural pest *D. suzukii* can provide valuable information regarding their management and control (Lee *et al.*, 2011a), while other *Drosophila* species may act as vectors for the transport of animals, such as nematodes, which can themselves have profound impacts on ecosystems (Kiontke and Fitch, 2013). To study these and other behaviors in a controlled laboratory setting requires a number of different and specific experimental apparatuses, whose creation can be an exercise in compromise. Off-the shelf arenas (petri dishes, vials, *etc*.) are rarely the optimal size or shape and are often difficult to modify (*e.g.*, the addition of entrances, dividers, or gates) due to their materials (*i.e.*, glass or brittle plastic). Adding to that the need to control opacity, color, optical qualities, or texture leaves the experimenter with the choice of making do with sub-optimal equipment or spending considerable resources constructing a custom fabricated item. With the availability and affordability and ease of desktop 3D printing equipment, however, this is no longer the case.

Consumer-level 3D printers and design software are revolutionizing many areas of scientific investigation (Chen, 2012, Lipson and Kurman, 2013, Chia and Wu, 2015). While previously only available in well-funded engineering labs, the recent availability of affordable units that can print in multiple types of plastic, and at sub-millimeter resolutions, means that researchers can quickly and easily design and print anything from replacement parts for larger devices to the purpose-built chambers needed for biological research, such as behavioral studies with *Drosophila*.

In this note we demonstrate the functionality of this on-demand, rapid prototyping and production system to support research into: 1) *D. suzukii*'s feeding preferences; and 2) *D. suzukii*'s ability to act as vectors for nematode dispersal. Each device could easily be adapted for use with other insect species.

Case study 1. *Feeding choice by* D. suzukii

Determining the characteristics that attract *D. suzukii*, a significant agricultural pest, to particular food sources may provide useful information for their control (Lee *et al.*, 2011a). The chambers designed and built for this experiment provide advantages over the classic population box (Dobzhansky, 1947; Lewontin, 1965; Caravaca and, Lei 2016). First, they allow for increased replication due to their size and affordability. Second, they provide for easier and more effective collection of flies that have chosen a treatment.

The chambers provide a simple two-choice environment (though they could easily be modified to provide more choices) that is small enough to allow full exploration by the flies, but still large enough that their choices are clearly evident. Further, flies can be effectively isolated with their choice without fear of losing specimens while capping jars or chasing them with a suck-tube.

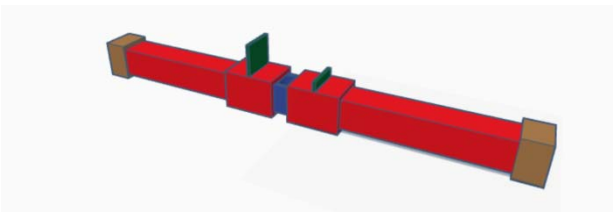

Figure 1. Rendering of the choice chamber. Gates (green) are shown in both the open and closed positions. The central addition port (where flies are added at the start of a trial) is shown here (in blue) before the gates would be joined.

The chamber (Figure 1), printed in transparent polylactic acid (PLA) plastic, consists of two tubes (25 mm × 25 mm × 140 mm) each with a removable cap at one end and a gate at the other. Food choices are placed in each of the capped ends and the tubes are joined by a connector with a port on top allowing for the addition of flies equidistant from both choices. The gates are then pushed together to close the port and seal the two tubes together. After the trial is finished the gates are dropped, sealing any flies into the tube with their choice.

Tests on this design were run using twenty flies per replicate (n = 20), with three different blueberry (BB) type comparisons (Table 1) alternating the sides on which each choice was located. Fake blueberries were made with pure pureed blueberries and fashioned into blueberry mimics. This technique, described elsewhere in this volume (McDevitt *et al*., 2018), allows for future studies of specific chemical and physical characteristics of berry food or oviposition choices. After 24 hours, the gates were dropped and the flies in each chamber counted.

Table 1. Treatment choices, average % (+/- SD) of flies in each treatment's side.

Choice 1	% near choice 1	Choice 2	% near choice 2
Thawed BB	87 (10)	Nothing	13 (10)
Fake BB	74 (14)	Nothing	26 (14)
Thawed BB	53 (16)	Fake	47 (16)

For these tests, individual chi-square analyses were run for each replicate with an assumption of no preference for either side. Thawed BB treatments were significantly preferred ($p < 0.05$) 18/20 times, while the fake blueberries were significantly preferred 13/20 times, and the fake *vs*. real trials found no significant difference between choices in any of the replicates.

These tests demonstrated to us that the chamber design was small enough for flies to find the food choices, that there were no inherent preferential regions leading to artefactual distributions (no side was preferred), and that flies could be successfully introduced into and isolated from the chambers where their food choices reside. While our PLA versions did not allow for visualization of flies during the trial, it is possible to have these printed in clear acrylic, which would permit real-time visual monitoring and recording of fly movement during the trials if appropriate.

Case study 2. Drosophila *as a vector for the external transport of nematodes*

Nematodes, such as the model organism *Caenorhabditis elegans*, have been well-studied in the lab but surprisingly little is known about their ecology (Kiontke and Sudhouse, 2006). Wild nematode species are often found associated with other animals, including insects such as *Drosophila,* where their attachment is thought to be a primary means for their dispersal to new ecological niches (Lee *et al.,* 2011b). When food is scarce and conditions unfavorable, *C. elegans* will enter into a developmental life stage called the dauer stage (Hu, 2007). Dauer larvae carry out an interesting dauer-specific behavior called nictation that involves the attachment of the worm to a substrate, such as fungi (Lee *et al.,* 2011b) and the waving of their heads. It has been shown that nictation leads to a higher incidence of the nematode attaching to an insect vector (Lee *et al.,* 2011b). However, much is not known about these interactions, specifically the frequency of attachment, rate of transfer, and any species-specific effects on that rate from either worms or their insect vectors. Such relationships may provide important information for biocontrol of *Drosophila*, nematodes, and other pests (Lee *et al.,* 2011a).

To determine the rate at which an individual fly would pick up, move, and drop off a nematode at a new location, we designed and printed small chambers that housed a single fly (*D. suzukii*) along with source and sink environments for the worms (*C. elegans*). The design provides clear viewing of the fly and the worms from above the chamber, while allowing illumination from the bottom, using a previously described imaging platform (Churgin *et al.,* 2017). This was done by using the parts of the petri dish on which the worms are grown, or carried to, as both the base (dish) and top (lid) of the chambers (Figure 2). The design and construction of the chamber provides for a new, clean viewing port for each replicate while providing fly-tight seals at both the top and bottom. A gate allows the fly's movement to be limited to the source side for a controlled amount of time before exposing them to the sink.

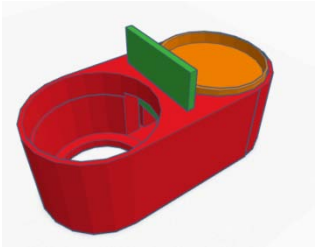

Figure 2. Rendering of the nematode vector chamber. Gate (green) is shown in the open position allowing the fly to move from the source to the sink side. The petri lid (orange) is shown on one side as it would sit, inverted, becoming the top window. The other side shows how the floor is open allowing light to pass up through the floor to backlight the worms for visualization. The actual chamber was printed in opaque black PLA.

Initial trials were conducted where ~7500 *C. elegans* dauer larvae were placed into the source side, which contained a 40 mm petri dish with nematode growth medium covered in medical gauze, a nictating substrate that was previously described (Lee *et al.*, 2011). A single *D. suzukii* fly was added to the source side for 1 hour, the gate was opened for ~10 minutes allowing for the fly to travel to the sink side of the device (which contained a fresh 40 mm dish of medium with no worms). After another hour, the number of worms on the sink side of the device was easily quantified as any worms present on the sink plate had to have been carried there by the fly. This was repeated for 7 trials. In only 1 of the 7 trials were worms transferred (two worms), suggesting that transfer is very infrequent, which is supported by another study (Lee *et al.*, 2011).

These initial experiments, using the custom chambers, illustrate that worms are carried by flies, and that the worms can be easily visualized on both the source and sink plates. Further, we found that worm dispersal via fly likely occurs in the range of single digits per fly per hour, at most. This low rate took place even in a chamber which should exacerbate the chances of this happening due to its small volume increasing the potential for contact between flies, worms, and substrates. This study is, however, in its infancy and has only looked at *D. suzukii*. Future experiments will alter the time the flies are exposed to the source, allowing for a temporal analysis of the frequency of transfer, and will compare different fly species to determine if these can affect the rate of transfer. Moreover, these experiments could be expanded to genetic studies using different fly species or *C. elegans* strains.

Discussion

Using easy to design software and a desktop 3D printer, we were able to custom-build effective research equipment that would otherwise have been impossible or too costly to produce. Using our Makerbot2 printer and free online design software (tinkercad.com), we were able to test many designs, often taking less than an hour from idea to production. Changes in gate configuration, sizes, shapes, opacity, color, and other parameters were tested, modified, and ultimately replicated for pennies a prototype (a $40 spool of PLA was more than enough to fuel the entire prototyping and production run of 20 choice chambers). We know of no other way that equipment such as this could have been developed and built as economically and quickly without this new technology. While our printer, a 5-year-old Makerbot2, is a high-end prosumer model (the latest version retails for $2,499), there are many less expensive models from a range of different companies (Makezine) that should be able to produce similar products, and even a model such as ours can pay for itself quite quickly.

Being able to set the dimensions of the equipment based on what was best for the experiment and not what supplies were available allowed us much more freedom, and likely resulted in better experimental results. While we continue to improve our designs, the current versions have been made available to use, modify, and print from the website thingiverse.com (items 2735670 and 2735695), or if researchers do not have access to a printer, the current designs can be produced by a third-party 3D production house such as shapeways.com (https://www.shapeways.com/shops/inator-devices), which will produce uploaded designs in a range of materials ranging from metals to various forms of plastic. We hope that the continued development of easy to use and affordable 3D printing will help drosophilists continue to learn more about these important, but often difficult to study organisms.

References: Caravaca, J.M., and E.P. Lei 2016, JoVE 109; Chen, A., 2012, Chronicle of Higher Education (www.chronicle.com/article/3-D-Printers-Arent-Just-for/134440); Chia, H.N., and B.M. Wu 2015,

J. Biol. Eng. 9: 4; Churgin, M.A, S.K. Jung, C.C. Yu, X. Chen, D.M. Raizen, Fang-Yen C. Elife 2017, May 31: 6; Cini, A., C. Ioriatti, and G. Anfora 2012, Bull. Insectology 65(1): 149-160; Dobzhansky, Th., 1947, Evolution 1: 1-16; Hu, P.J., 2007, Wormbook Aug 8: 1-19; Kiontke, K., and D.H.A. Fitch 2013, Current Biology 23: R862-864; Kiontke, K., and Sudhouse 2006, Wormbook Jan 9: 1-14; Lipson, H.M., and D. Kurman 2013, *Fabricated: The New World of 3D Printing*. Indianapolis, IN: Wiley; Lee, J.C., D.J. Bruck, A.J. Dreves, C. Loriatti, H. Vogt, and P. Baufeld 2011, Pest Manag. Sci. 67: 1349-51; Lee, H., M.K. Choice, D. Lee, H.S. Kim, H. Hwang, H. Kim, S. Park, Y.K. Paik, and J. Lee 2011, Nat. Neurosci. Nov 13: 107-12; makezine.com/comparison/3dprinters/; McDevitt, D., S. McRobert, and J. Fingerut 2017, Dros. Inf. Serv. 100: this issue.

Culture medium for flower-breeding drosophilids.

Larroza, A., G.V. Mello, and J. Cordeiro. Departamento de Ecologia, Zoologia e Genetica; Instituto de Biologia; Universidade Federal de Pelotas, RS, Brazil. E-mail: juliana.cordeiro@ufpel.edu.br

Despite more than 6,800 species of drosophilids are known (Bächli, 2017), very few species are easily maintained in the laboratory and could be used as research models. Among the reasons, it is relatively difficult to keep some lineages, particularly when they have specific developmental requirements. Flower-breeding drosophilids are one of these species that could not be maintained in the laboratory and extremely depends on living flowers. In this context, the recipe presented below was developed to rear flower-breeding drosophilids that do not grow in already described medium, such as the ones from Bizzo *et al.* (2012), Markow and O'Grady (2006), Marques *et al.* (1966), Schmitz (2016), and Vaz *et al.* (2014).

This recipe was developed and has been used in the *Laboratory of Genetic Diversity and Evolution* of *Universidade Federal de Pelotas* (Rio Grande do Sul, Brazil), prepared with flower extracts from *Brugmansia suaveolens* (Solanaceae) and *Ipomoea alba* (Convolvulaceae). However, it can be performed using extracts of other flower species, or even from mushrooms. It proved to be successful for raising *D. bromelioides*-like species (new species not yet properly described, named '*tipo III*' by Schmitz (2010) belonging to the *Drosophila bromeliae* group, from *Drosophila* genus) and *D. denieri* (belonging to the *Phloridosa* subgenus from *Drosophila* genus). Both species depend on flowers to develop their life cycle (Brncic, 1983; Schmitz, 2010). In our tests the lineages were maintained until F4 generation, in good performance.

The preparation of the medium uses common ingredients and the flowers can be kept in the freezer until their use, overcoming the natural periodicity of the blooms. Also, the equipment used is accessible making the routine preparation of this medium very cheap and easy.

Medium ingredients

0.22 g of agar
0.45 g of sugar
0.02 g of dry yeast biological
0.01 g of methilparaben
10ml of distilled water
1 macerated flower

Procedure

- Mix agar and 5 ml of distilled water and wait 10 min
- Add the remaining distilled water, the sugar, and the dry yeast
- Mix everything and boil on the microwave three times for 1 min