Observation Hive Marking Apparatus (OHMA): an inexpensive method for mass-marking honey bees in observation hives

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Introduction

Hagler and colleagues (2011) developed an effective way for mass-marking honey bees from multiple apiary locations. The method, however only works with Langstroth hives. Since honey bee research is often conducted with smaller observation hives, we made modifications to the method for mass-marking bees from these smaller hives. We used this modified approach to track hive-specific foraging preferences. By using multiple hive-specific colors, we were able to count the number of bees from each hive at each experimental foraging location (Figure 1). Using observation hives allowed us to easily track hive-specific micronutrient preferences (Bonoan *et al., in prep*).

Dust-Marking Insects

Scientists have been marking insects since the 1920s beginning with simple paints, dyes, and stains (Hagler & Jackson, 2001). Recent developments in insect marking include protein marking (e.g. ELISA) (Hagler & Jackson, 2001), genetically engineered marking using transposable elements (Hagler & Jackson, 2001), and radiofrequency identification (Schneider, Tautz, Grünewald, & Fuchs, 2012). Such recent developments are more accurate but are expensive and not always necessary.

Here we describe a method using Day-Glo's fluorescent ECO Pigments (Day-Glo Color Corporation) which are visible to the naked eye (Figure 2), and free of harmful chemicals (such as formaldehydes and heavy metals). ECO Pigments come in a variety of colors—thus, multiple hives can be individually marked—and do not have any negative effects on honey bees (Hagler, Mueller, Teuber, van Deynze, & Martin, 2011). Dust marking can also be done by puffing the insect with a cloud of colored dust or by tumbling the insects in a dust-filled container (Hagler & Jackson, 2001) however, both require handling of the insects. In both this modified method and the Hagler *et al.* (2011) method, the insects self-mark and thus are not handled by an experimenter.

The Method

Our observation hives are housed in a shed with an exit tube running through the wall to allow bees to forage freely (Figure 3). Before making the observation hive marking apparatus (OHMA), the bees must have a landing strip. The exit tube needs to be flush with the outside wall so that the OHMA can be attached to the exterior wall on either side of the tube (Figure 3).

Other than the marking powder, all materials to create the OHMA are available at the hardware store. The device itself is constructed with vinyl mesh, a hot glue gun, and heavy duty Velcro (Scotch Extreme Fastener, 1"X10'). First, we created a mesh satchel for the powder by cutting a 4 cm X 8 cm piece of mesh, folding the mesh in half, and hot gluing the two ends together. At this point, we had a 2 cm X 8 cm mesh satchel with an opening on top for loading the powder. Then, we cut two pieces of Velcro (about 1 cm X 2 cm) and hot glued one piece to each end of the mesh satchel. Next, we cut two more pieces of Velcro (about 1 cm X 2 cm) and stuck them to the wall on either side of the exit tube. We used small picture hanging nails to further secure the Velcro in place. The Velcro allows for the OHMA to be applied and removed multiple times.

When time to mark the bees, each satchel was filled with about 1 tablespoon of marking powder. The satchel was then secured onto the Velcro so it blocked most of the exit hole. This requires the bees to brush up against the mesh, and thus get dusted with the colored powder, as they leave and enter the hive. The bees quickly adjust to the constrained exit/entrance tube. This

is where the landing strip is important—the bees require a place to land and then crawl into the hive under the OHMA, or else the bees cannot get back in. At the end of the experimental period, the OHMAs can be removed to allow the bees to come and go freely.

Since we were only interested in identifying bees by hive for a short foraging time, this method worked perfectly (Figure 1). The foraging area where we observed the marked bees was about 100 meters away from our observation hives; bees remained marked through observations. Although 100 meters is not far for a forager, neighbors with gardens and students on campus observed marked foragers (anecdotal evidence) beyond 100 meters. The bees tend to mostly clean themselves off before they returned to the hive, however there are parts of their body that they cannot reach and remain marked. In some cases, the pigment did make it into the wax comb but there was never dis-coloration of the stored honey or pollen.

The OHMA can be used to mass- and self-mark insects wherever there is a small exit/entrance hole. For example, OHMAs can be situated near floral-visitation sites, insect bait stations, insect traps, and at natural nest entrances (Hagler & Jackson, 2001). With honey bees in particular, OHMAs can be used to investigate nuanced questions regarding honey bee behavior both inside and outside the observation hive (Table 1, and references therein). Outside the hive, this method allows for identification and tracking of unique hives. Inside the hive, this method allows for an easier way to visually follow bees upon their return to the hive (Ikeno et al., 2008).

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References

Allen, D. M. (1956). The behaviour of honeybees preparing to swarm. *British Journal of Animal Behaviour, 4*(1), 14-22.

Božič, J., & Valentinčič, T. (1995). Quantitative analysis of social grooming behavior of the honey bee *Apis mellifera carnica*. *Apidologie, 26*(2), 141-147.

Danner, N., Haertel, S., & Steffan-Dewenter, I. (2014). Maize pollen foraging by honey bees in relation to crop area and landscape context. *Basic and Applied Ecology, 15*(8), 677-684. doi:10.1016/j.baae.2014.08.010

Degrandi-Hoffman, G., & Chambers, M. (2006). Effects of honey bee (Hymenoptera: Apidae) foraging on seed set in self-fertile sunflowers (*Helianthus annuus L*). *Environmental Entomology, 35*(4), 1103-1108. doi:10.1603/0046-225X-35.4.1103

Farina, W. M. (1996). Food-exchange by foragers in the hive: a means of communication among honey bees? *Behavioral Ecology and Sociobiology, 38*(1), 59-64. doi:10.1007/s002650050217

Fefferman, N. H., & Starks, P. T. (2006). A modeling approach to swarming in honey bees (*Apis mellifera*). *Insectes Sociaux, 53*, 37-45.

Hagler, J. R., & Jackson, C. G. (2001). Methods for marking insects: current techniques and future prospects. *Annu Rev Entomol, 46*, 511-543.

Hagler, J. R., Mueller, S., Teuber, L. R., van Deynze, A., & Martin, J. (2011). A method for distinctly marking honey bees, *Apis mellifera*, originating from multiple apiary locations. *Journal of Insect Science, 11*(143).

Ikeno, H., Okada, R., Ohashi, M., Kimura, T., Akamatsu, T., & Ito, E. (2008). A movement index for evaluation of honeybee activity in the observation hive. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology, 151*(4), 456-456. doi:10.1016/j.cbpb.2008.09.035

Pernal, S. F., & Currie, R. W. (2001). The influence of pollen quality on foraging behavior in honeybees (*Apis mellifera L.*). *Behavioral Ecology and Sociobiology, 51*(1), 53-68. doi:10.1007/s002650100412

Rangel, J., & Seeley, T. D. (2008). The signals initiating the mass exodus of a honeybee swarm from its nest. *Animal Behaviour, 76*, 1943-1952. doi:10.1016/j.anbehav.2008.09.004

Schneider, C. W., Tautz, J., Grünewald, B., & Fuchs, S. (2012). RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*. *PLoS One, 7*(1), e30023. doi:10.1371/journal.pone.0030023

Schuerch, R., Couvillon, M. J., Burns, D. D. R., Tasman, K., Waxman, D., & Ratnieks, F. L. W. (2013). Incorporating variability in honey bee waggle dance decoding improves the mapping of communicated resource locations. *Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology, 199*(12), 1143-1152. doi:10.1007/s00359-013-0860-4

Steffan-Dewenter, I., & Kuhn, A. (2003). Honeybee foraging in differentially structured landscapes. *Proceedings of the Royal Society B-Biological Sciences, 270*(1515), 569-575. doi:10.1098/rspb.2002.2292

Tanner, D. A., & Visscher, K. (2006). Do honey bees tune error in their dances in nectar-foraging and house-hunting? *Behavioral Ecology and Sociobiology, 59*(4), 571-576. doi:10.1007/s00265-005-0082-z

Unger, P., & Guzman-Novoa, E. (2012). Honey bee (Hymenoptera: Apidae) guarding behaviour may not be a rare task. *Canadian Entomologist, 144*(4), 538-541. doi:10.4039/tce.2012.58

123 **Table 1.** Types of questions that can be investigated using the OHMA.

124 **Figure 1.** Mass-marked honey bees from the yellow, pink, and orange hives foraging for
125 micronutrients at our experimental foraging area. Photos: Rachael E. Bonoan

126 **Figure 2.** A forager dusted with yellow marking powder. Photo: Rachael E. Bonoan

127 **Figure 3.** Schematic of the observation hive set-up.