

Please print these instructions for reference.

Healthy Bee 2020 Project

Establishing the Deformed Wing Viral (DWV) diversity across the USA

Principal Investigator, Prof. Stephen J. Martin, School of Environmental & Life Sciences, University of Salford, Manchester, M5 4WT, UK (s.j.martin@salford.ac.uk)

Cooperating Investigator, Randy Oliver, Scientific Beekeeping, 14744 Meadow Dr. Grass Valley, CA 95945 (randy@randyoliver.com), (530) 277-4450

Dear cooperating beekeeper,

Thank you for volunteering to collect bee samples for this project. If you have any questions, please contact Randy by email or phone (mornings best).

Objective of this Research

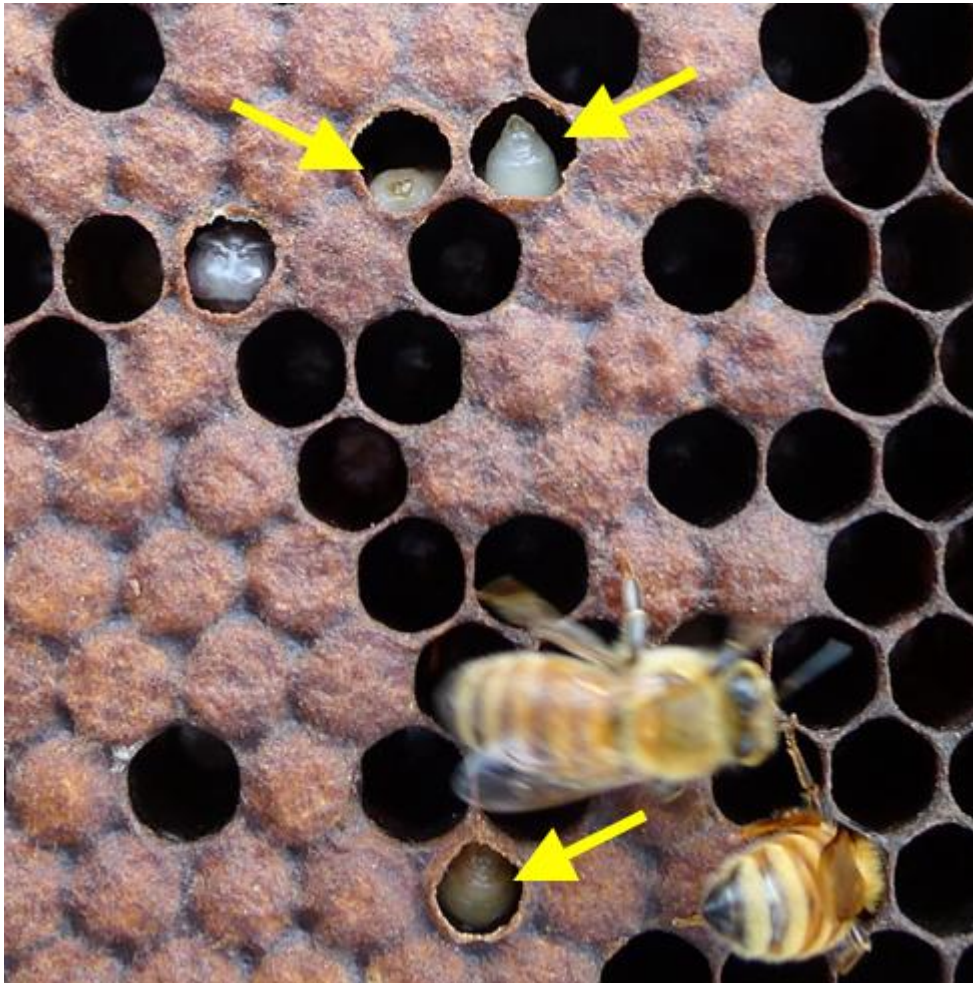
Almost all honey bees are now infected with Deformed Wing Virus (DWV) but the impact on a colony's health is highly variable. In part this is because DWV exists in many types (strains)--currently A, B and C forms of DWV have been described. Each different type of DWV has a different impact on the honeybee colony.

In the vast majority of cases, if varroa is not controlled the colony will eventually succumb to the virulent DWV type A strain. However, some honeybee populations have evolved various mechanisms to resist varroa and the impact of viruses. Scientists are trying to understand these mechanisms. One interesting mechanism discovered in the UK is known as Super Infection Exclusion--when a non-lethal strain of a virus 'DWV type-B', out competes the more lethal type-A. In the order to improve our understanding of the distribution of the different types of DWV in different honeybee populations, we have been funded by Project Apism to conduct a DWV survey of the USA honeybee population.

We interested in finding to what degree different strains of DWV contribute to the survival of colonies that are unmanaged for varroa (survivor hives and ferals), and how that compares to the DWV strains in treated hives or hives dying from the varroa/virus complex.

We're especially interested in:

- Samples of adult bees from survivor colonies that have fairly high mite counts (>15 mites in an alcohol wash of 1/2 cup of bees) yet exhibit a healthy brood pattern (no dying pupae)--this may indicate the presence of a benign strain of DWV.
- Samples of adult bees, and especially of dying propupae (in a separate tube), from mite-infested colonies starting to exhibit signs of Parasitic Mite Syndrome. Propupae have no eyes, and if dying, will be uncapped and starting to turn gray. We're curious as to which viruses are killing propupae prior to a mite feeding on them.



Propupae at yellow arrows. Propupae are larvae about to pupate, and thus have no eyes formed yet.

- We also need some reference samples of adult bees from "normal" apiaries not selected for survivor stock (perhaps from a neighbor).

The Sample Kit

Enclosed in this pack are:

- 50mL collection tubes containing ~20mL of 100% ethanol (denatured alcohol).
- A silver fork for removing excess bees from the sample tube.
- Labels and a pencil.
- A zipper lock bag to enclose the tubes (in case of leakage).
- An addressed and stamped return box.

Sampling

Please collect 20-30 bees from the same colony into each collection tube.

Collect no more than 5 colonies per apiary (honeybee population). If you can collect from other locations, please ask for additional sampling kits.

If possible collected the bees from the surface of the brood frame, but bees at the entrance are also fine if you don't wish, or can't get to the brood frames e.g. a feral colony.

The bees can be placed directly into the ethanol, or chilled first, it is up to the beekeeper.

This kit contains vials containing 20 mL of denatured alcohol (undrinkable). In general, the easiest way to take a bee sample is by holding a frame of bees vertically, and gently drag the opened vial downward over the bees' bodies. This will cause them to release their grip and fall into the vial (see photos below).



Collecting bee samples. Drag the opened vial downward, gently rubbing the bees' bodies. They will release their grip and fall into the alcohol. We only need 20-30 bees per vial.

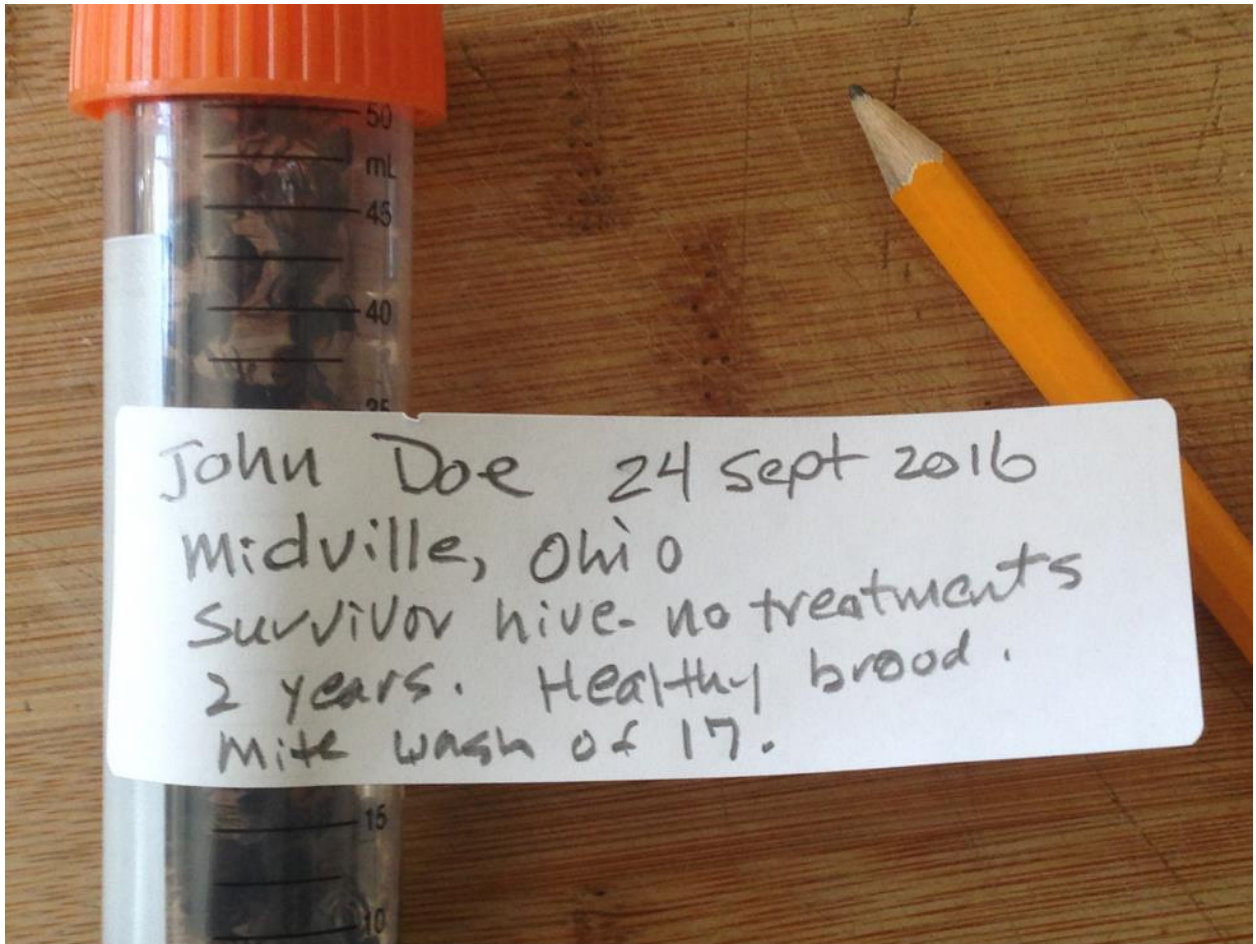
Please take a sample of 20-30 bees, but no more (as any additional bees will dilute the alcohol's preservative effect). You can take the sample, and remove extra bees immediately afterwards, using the enclosed silver fork (it's not real silver, but you can keep it anyway). To estimate your count, hold the vial upright and jiggle it, allowing the bees to gently settle—do not pack them down. 25 bees will just reach the 20 mL mark. 30 will just reach the mark between 20 and 25 mL (see photo below).



The above photo shows a vial with 30 bees settled to the 20 mL mark. In any case, there should be some free alcohol above the bees. Then seal the lid snugly so that it doesn't leak in transit.

Please label (in pencil, with included labels, see photo below) each vial as to:

- Your name
- Date of sampling
- Approximate location
- Brief history of the hive (colony/apiary free of mite treatments for x years, treated hive from commercial bee stock, feral colony, colony dying from varroa/PMS)
- Other relevant info: mite count in 300 bees, health of brood, condition of colony, etc.



Please label each vial as shown, writing in pencil only (in case of alcohol leakage). Wrap the label around the vial.

Please store the samples in your refrigerator (NOT the FREEZER) until you are ready to ship them.

Return Shipping

Place all vials into the zipper lock plastic bag, and return to Randy in the postage-paid, preaddressed box. You can leave the box in your mailbox for pick up.

Thank you for contributing to bee research!

Randy and Stephen