

National Honey Board Project Report: Evaluating the Potential Benefits of Native Prairie Flowers for Honey Bees

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Introduction:

To help determine which native prairie flowers can contribute to honey bee colony nutrition and productivity, we collected pollen and unripe honey from colonies every two weeks during the summer and fall. Our colonies were located in various locations around the Twin Cities area: at Werth Farm and Prairie Restoration in 2013 and at Belwin Conservancy in Afton and the Carleton College Arboretum in Northfield in 2014. In 2015, we continued collecting at Belwin Conservancy and focused on decoding waggle dance communications among honey bee foragers about high-quality flower patches in and around Belwin Conservancy.

Reference collection:

We use slides of pollen collected from flowers as a reference for comparison with grains in the pollen loads and stored nectar of bees. We have collected, identified, and made slides of pollen from 90 species of flowers in and around our native prairie sites. We have also made progress in photographing and describing pollen from Minnesota native flower species in a large pollen reference collection housed at the University of Minnesota. We have photographed over 200 species so far. We recently established connections with the LacCore Facility (<https://tmi.laccore.umn.edu/>) and the Bell Museum of Natural History (<http://bellatlas.umn.edu/>) at the University of Minnesota so that we can begin sharing our photos and descriptions online with other bee researchers and the public.

Pollen Analysis:

In addition to making slides of our pollen samples under the microscope, in January we collaborated with Deborah Iwanowicz and Scott Cornman at the United States Geological Survey to sequence the DNA in our pollen samples using the protocol that they recently developed (Cornman et al. 2015). The sequencing data does not give us proportions of different species, but it will help us to confirm our pollen identifications and identify pollen grains more specifically (ex. to species rather than simply to genus). The sequencing results have already helped us to identify unknown pollen types; for example, it helps differentiate species within families of plants that have similar pollen structures, such as within the asters and within the mustards. We are working to determine gaps in online databases of plant DNA sequences that we can fill by adding sequences of unrepresented native prairie species to achieve even better species-level resolution.

Overall, the results indicate a large contribution of native tree pollen in the spring and summer (ex. *Acer*- maple, *Tilia*- basswood, *Rhus*- sumac), non-native Fabaceae pollen in the summer and fall (ex. *Trifolium*- white/red clover, *Melilotus*- sweet clover, and *Lotus corniculatus*- birds-foot trefoil), and native and non-native aster pollen in the late summer and fall (ex. *Ambrosia*- ragweed, *Arctium*- burdock). In addition, we have identified several native prairie plants that contribute to colony food stores, including *Veronicastrum virginicum* (culver's root), *Impatiens* (touch-me-nots), *Dalea purpurea* (purple prairie

clover), *Allium* (wild onion/chives/garlic), *Solidago* (goldenrods), and *Symphyotrichum* (asters).

Nectar Analysis:

We are finishing microscope identification and validation using the sequencing results. Results so far indicate contributions from non-prairie species, including sweet and white clover, basswood, *Rhamnus* (buckthorn), *Raphanus* (radish), *Glycine max* (soybean), *Brassica nigra* (black mustard), and *Taraxacum* (dandelion), and several native species, including touch-me-not, *Helianthus* (sunflower), prairie clovers, and goldenrod/asters. Despite the unexpectedly low numbers of mint pollen grains, mints such as those in the genera *Agastache* (giant/anise hyssop) and *Pycnanthemum* (mountain mint) may still be an attractive source of nectar. According to Bryant and Jones (2001), species in the mint family tend to be under-represented in honey relative to species in other families. We are currently setting up experiments to test the attractiveness of several native species, including mints using observations of visitation and waggle dance mapping.

On average, over 75% of the DNA sequence reads from our unripe honey samples were from non-native sweet clovers and white/alsike clovers. The table below shows the top ten native prairie species/genera based on their proportions of DNA sequence reads per sample (note: this list may change when we finish integrating the light microscopy results with the DNA sequencing results):

| Common name | Scientific name | Native | Maximum % | Average % |
|-----------------------|-------------------------------------|--------|-----------|-----------|
| Cinquefoils | <i>Potentilla</i> | Maybe | 13.2 | 1 |
| Purple prairie clover | <i>Dalea purpurea</i> | Yes | 2.9 | 0.3 |
| Heartleaf aster | <i>Symphyotrichum cordifolium</i> | Yes | 2.5 | 0.4 |
| Lupines | <i>Lupinus</i> | Maybe | 1.9 | 0.2 |
| Goldenrods | <i>Solidago</i> | Yes | 1.3 | 0.4 |
| Sunflowers | <i>Helianthus</i> | Yes | 1.3 | 0.4 |
| Hyssop | <i>Agastache</i> | Yes | 0.7 | 0.05 |
| Smartweeds | <i>Persicaria</i> | Maybe | 0.7 | 0.05 |
| New England aster | <i>Symphyotrichum novae-angliae</i> | Yes | 0.3 | 0.1 |
| Canada anemone | <i>Anemone canadensis</i> | Yes | 0.2 | 0.02 |

Dance decoding results:

We continue to videotape honey bee behavior in three observation hives at Belwin Conservancy. Last year we also trained honey bee foragers to dance for feeders at known distances so that we can calculate distances from dances to unknown flower patches. So far, we have decoded 500 dances (a little over a third of the total). Of these, roughly 150 were dances for pollen sources in which we collected the dancer's pollen loads and identified them under the microscope. We have also received R code from Roger Schürch at the University of Sussex (Schürch et al. 2013) and modified this code in order to plot the dances. We will not make final analyses of forager preferences until we have decoded all dances, but preliminary analyses indicate that foragers did dance for both pollen and nectar sources within reconstructed prairies. Surprisingly, some of the pollen foragers that danced for sites within prairies had collected pollen from non-native flowers, such as red clover pollen (flower surveys in these prairies indicate that they contained native and non-native flowers).

Conclusion:

We have made progress in expanding the diversity of reference images used to identify bee-collected pollen grains. We are in the process of finishing microscopy work and comparing our new DNA sequencing results to our results from microscopy. **These results will allow us to finalize a list of native prairie flowers that are attractive to honey bees and assess their total contributions to colony food stores.** We also videotaped waggle dances, collected and identified pollen loads from dancing foragers, and we are in the process of decoding and mapping these dances. When finished, these data will help us to understand whether honey bees prefer flower patches in prairies, and which native prairie flower species they advertise within prairies (which species and patches do they consider the best resources?).

References:

- Bryant VM Jr., Jones GD (2001) The R-values of honey. *Palynology*. 25: 11-28
- Cornman RS, Otto CR V, Iwanowicz D, Pettis JS (2015) Taxonomic characterization of honey bee (*Apis mellifera*) pollen foraging based on non-overlapping paired-end sequencing of nuclear ribosomal loci. *PLoS ONE*. 10: e0145365
- Schürch R, Couvillon MJ, Burns DDR, Tasman K, Waxman D, and Ratnieks FLW (2013) Incorporating variability in honey bee waggle dance decoding improves the mapping of communicated resource locations. *J Comp Physiol [A]*. 1999: 1143-1152