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Research Article

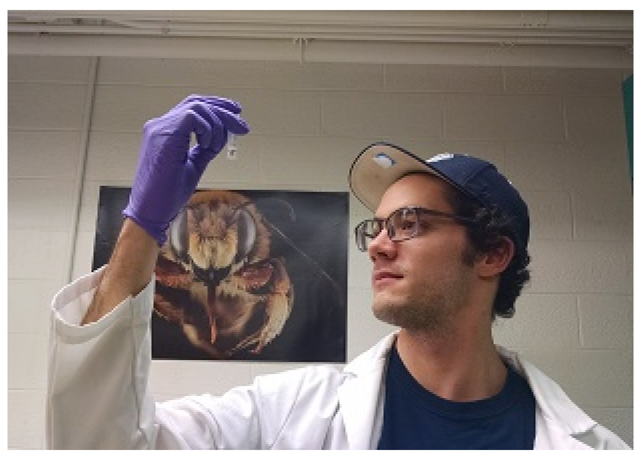
Comparison of Cuticular Hydrocarbons in Three Populations of the Carpenter Bee “Ceratina calcarata” to help Understand their Role in Social Evolution

—Sean Lombard (Editor: Rosie Donegan)

You could say it was fate, you could say that it was destiny, or you could say it was just *meant to bee*. That pun was intended, though my introduction to bees happened quite unintentionally when, as a freshman in the fall of 2012, I stumbled upon the University of New Hampshire (UNH) Bee Lab and have been engrossed in bee research ever since. Under the direction of my mentor, Dr. Sandra Rehan, assistant professor of biological sciences, my studies have been focused on the small carpenter bee *Ceratina calcarata*, which is about the size of a carpenter ant and can range in color from blue-green to dark teal.

Most people associate bees with honey, but strangely, the only bees that make honey are those of the species *Apis mellifera*, which was introduced from Europe. So if only European honeybees make honey, why study native bees like *C. calcarata*?

Recently there has been rising concern that the activities of humans have had a negative effect on native bee populations, and that many bee populations in North America are in decline. This is important because bees provide global pollination services estimated at roughly $160 billion U.S. dollars annually (Gallai et al., 2009). Native bees are especially good at pollinating certain plants, like tomatoes and blueberries, because of their capacity to sonicate, or vibrate at certain frequencies to loosen the pollen and make it easier to collect, something that honeybees are incapable of doing (Greenleaf & Kremen, 2006). By studying native bees, we can hopefully begin to change our lifestyles to ensure their survival and well-*beeing* in order to ensure, in turn, our survival and well-being.



The author examining a *Ceratina calcarata* specimen in the UNH Bee Lab (*Photo by Kieran Lombard*).

The purpose of my research over the past two summers has been to analyze the composition of the cuticular hydrocarbons (CHCs) found on the surface, or cuticle, of *C. calcarata*. CHCs are chains of hydrogen and carbon molecules, usually fifteen to twenty carbons long, in different formations. They are thought to have initially evolved to prevent water loss, and then became part of chemical communication among individuals. Communication among insects plays a large role in their behavior and is critical to the development of complex social systems. Learning more about the role of CHCs is one small but important part in understanding how these systems evolved—and how to protect them.

During the summer of 2014, on a Research Experience Apprenticeship Program (REAP) scholarship, I had analyzed the CHCs of a New Hampshire population of *C. calcarata.* The following summer I was funded by a Summer Undergraduate Research Fellowship (SURF) to analyze and compare the CHC compositions of populations of *C. calcarata* from Missouri and Georgia along with those from New Hampshire. My goal was to find out if the CHC compositions differed significantly among these three populations. Our prediction was that CHC composition would vary by population.

Insect Societies

The complexities of insect interaction can vary greatly from simple solitary behaviors, in which a mother creates a nest by herself, lays eggs, and has no further interaction with her offspring, to the dynamic eusocial colonies comprising queen mothers and thousands of worker daughters as seen in the honeybee. For my research the most important characteristics of eusocial bees are the presence of a caste system, the presence of multiple generations of offspring, and the reproductive division of labor (Wilson, 1971; Michener, 1974). None of these characteristics are exhibited by solitary insects. In a caste system, each bee has its own specific role; for instance, a worker bee forages for pollen, and a queen bee monopolizes reproduction. Having multiple generations within one nest implies that there must be interaction between offspring and parent. Finally, reproductive division of labor is exemplified by the inability of non-queen bees to reproduce, either by physical assertion of the queen or by the secretion of pheromones like CHCs.

C. calcarata is classified as a subsocial bee. Subsociality is thought to be the stepping stone between solitary and eusocial behaviors, exhibiting some traits found in both. The display of intermediary behavior exhibited in *C. calcarata* makes the species ideal to study when asking about the process of social evolution from solitary to eusocial behaviors. For the most part, *C. calcarata* behaves like a solitary bee: the female will build her nest by herself, and lay her eggs. However, the mother then interacts with the offspring, cleaning the eggs as they hatch and develop into adult bees. The mother also intentionally withholds food from the eldest daughter so that she is dwarfed in size relative to her mother and sisters. The mother then forces this dwarf-eldest daughter to forage for pollen for her siblings so that they may survive the winter. The dwarf-eldest daughter does not survive the winter and is, therefore, unable to reproduce (Rehan & Richards, 2010). Both the interaction of a mother with her offspring and her manipulation of the dwarf-eldest daughter are

The small carpenter bee, *Ceratina calcarata* (*Courtesy Dr. Sandra Rehan, UNH Bee Lab*).

reminiscent of eusocial colonies. By studying *C. calcarata*, we hope to gain insight into the evolutionary history of eusociality from solitary ancestors.

Analyzing and Comparing Three Bee Populations

C. calcarata occupies a broad range of habitat from as far south as Georgia to as far north as New Hampshire. During the summer of 2015, nests were collected from Missouri and Georgia. One of the postdoctoral researchers in the lab, Sarah Lawson, collected the nests in Missouri for me, and Dr. Rehan collected those from Georgia. *C. calcarata* build nests in the dead branches of pithy plants like sumac, rose, and raspberry. Once a bee has found a suitable twig, it will hollow out the soft middle, or pith, of the plant to form its home. Collecting these nests means getting up before the sun rises to ensure the bees have not begun foraging. Once a nest is discovered in the field, the entrance is blocked with tape, the stem is clipped off at the base of the branch, and the nest is then stored on ice to ensure that none of the residents wake up. The bees flew back to UNH in a dry shipper (ironically requiring an airplane to fly in this instance). We processed all of the collections by splitting the nests open, catching and freezing the bees in liquid nitrogen, and measuring the nest sizes. In total, twelve females were collected from Missouri and eleven from Georgia. Ten specimens collected the previous year in New Hampshire were re-analyzed.

Next, I performed the CHC extractions by soaking the samples in pentane, a non-polar solvent in which the long hydrocarbon chains easily dissolve. The extracts were analyzed with gas chromatography and mass spectrometry to identify which CHCs were present on each individual. Other measurements that I performed were to control for different factors among the bees. These factors were age, size, and reproductive status. The purpose of these measurements was to ensure that drastic morphological differences among the populations of bees were not the reason for any significant differences in CHC composition. (For instance, if all of the bees from Georgia were much larger than bees from Missouri and there are drastic differences between the CHCs of both populations, there might be another factor involved in the CHC variation than just differences in the population's location). Wing wear, a measure of how ragged the wings are on a scale from zero to five, is used as a proxy for overall foraging effort and relative age. I also measured the head width of each of the bees because it is directly proportional to the overall size of the bee. The ovarian dissections and measurements to ascertain reproductive status were performed by Alona Brosh, another member of the Bee Lab who has mastered the technique.

Research Results and Extrapolation

Analysis of the CHC composition has shown much chemical similarity between the three populations. Many of the same CHCs are present in all three populations, but the relative concentrations of some CHCs differ significantly. A linear discriminant analysis (LDA) of all of the CHCs and specimens showed that the chemical composition did group individuals into the populations from where they were collected. Figure 1 shows the overall similarity of complex chemical profiles by grouping individuals based on relative chemical composition.

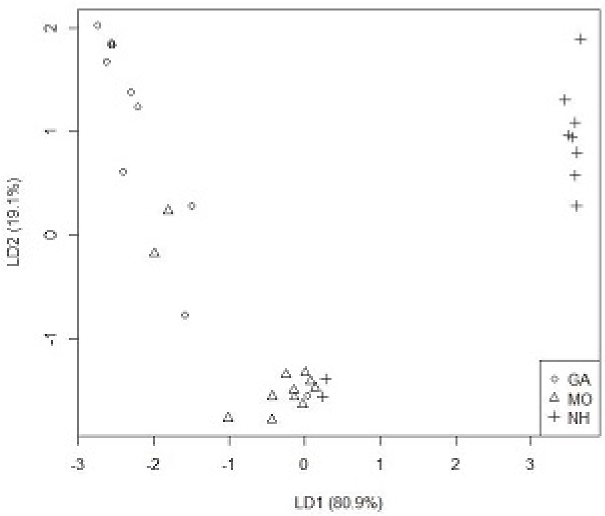


Figure 1: Linear discriminant analysis showing the variation of CHC profiles across three populations. Circles represent individuals from Georgia (GA), triangles are from Missouri (MO), and plus signs are New Hampshire (NH) bees. *Click on figure to enlarge.*

These results affirm the prediction that the CHC composition varies by population, though there does appear to be some overlap across populations. Currently members of the Bee Lab and I are analyzing these same populations using DNA sequencing techniques to glean the relative genetic relatedness among individuals from each population.

The CHC variation observed in our results suggest that chemical composition changes with latitude. The populations collected all occupy different climatic regions and possibly vary in their CHC composition in response to adaptation to local temperatures. I found that southern populations have longer chain CHCs, likely to prevent water loss in hot and humid conditions, and northern populations have shorter chain CHCs, probably due to the cooler climate. Because the populations are so isolated from one another, it is also possible that genetic variation could contribute to differences in CHC expression. Finally, the behavioral patterns of bees collected from Georgia and Missouri are likely to be different from New Hampshire, and could also explain some of the observed CHC variation, especially if the CHCs which vary are being used to guide interactions within the population, much like the pheromones of eusocial insect societies.

More Bees in my Future

Over the past year I have found myself in an even deeper realm of research than I was introduced to during my first year in the Bee Lab. My role as a researcher has shifted from that of an inexperienced worker to one of deeper understanding of and greater intellectual contribution to the outcome of the project. I am very excited by the results of this research, and I hope to continue studying *C. calcarata*, especially the populations in Georgia and Missouri. These populations are in lower latitudes with longer, warmer, summers, and are less limited by a short foraging season than *C. calcarata* in New Hampshire. Preliminary evidence suggests that the bees in Georgia exhibit bivoltinism, or the completion of two reproductive cycles in one year. If this is indeed the case, it is likely that the behavioral interactions of southern populations are more complex and may require different CHCs for more elaborate communications.

One of the most frustrating and wonderful parts of life is the knowledge that there will always be mysteries and a great unknown. This is exemplified by the results of my research and by my experiences as a researcher. Coming out of the REAP project with a lot of information about *C. calcarata* led to more questions than answers, as this project has done also. Similarly, though I made the mistake of thinking that I understood all of the general aspects of research after this SURF project, I have since realized that even after two years of experience, I am merely scratching the surface.

This research experience would not have been possible without the generous donations made by Mr. Dana Hamel and Dr. Joyce Boyd to UNH undergraduates through the Summer Undergraduate Research Fellowship (SURF) program, so I thank them for their generous support. The Hamel Center is run through the hard work of its members, who have been wonderful during my time as an undergraduate, including Dr. Paul Tsang, Dr. Molly Doyle, and Peter Akerman (to name a few). Finally, I thank Dr. Sandra Rehan for her wisdom and leadership during this and other projects as well as all the other wonderful members of the Bee Lab.

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Read Sean's commentary on his REAP experience, "Beehavior and Beyond: Realizations in Research," in the 2015 issue of *Inquiry*. REAP is an award program of the Hamel Center for Undergraduate Research for highly motivated freshman after completing their first year at UNH. They carry out a research project under the supervision of a UNH faculty member.

Author and Mentor Bios

Sean Lombard hails from Dover, New Hampshire and is majoring in biomedical science: medical and veterinary sciences. A member of the University Honors Program, Sean will graduate in 2017 with a bachelor of science. In the summer of 2014 he conducted research in the UNH Bee Lab as part of the Research Experience and Apprenticeship Program (REAP) and continued it the following summer, funded by a Summer Undergraduate Research Fellowship (SURF). His research activities with the Bee lab have taken him as far from home as Australia. He believes that "anything and everything is unique and fascinating. Research is a fun experience as a whole, and much like life, it has its ups and downs." Sean hopes to continue conducting research throughout his time at UNH, before following a path in clinical or research medicine.

Dr. **Sandra Rehan** is an assistant professor of biological sciences at the University of New Hampshire. She specializes in animal behavior, comparative genomics, and social evolution. She is a seasoned field biologist, having studied native bees and their social behavior for over ten years in North and Central America, Africa, Asia, Australia, and across the South Pacific. As a result, she is keenly interested in the environmental factors selecting for social behavior and considers Sean's research an important contribution to our understanding of chemical communication and ecology. In her two years of teaching at the University of New Hampshire, Dr. Rehan has mentored twenty undergraduates on a variety of topics, and Sean is her first *Inquiry* author. "Learning to write for *Inquiry* is a great opportunity for undergrads of all disciplines," she said. "It is particularly valuable for biologists to learn how to communicate their research to a broader audience."