Colony collapse disorder has beekeepers and researchers bumbling for a solution. What's buzzing in the Centre for High-Thoughput Biology? *An interview with Marta Guarna*

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Some may wonder about the relevance of fuzzy, innocent honey bees, but honey bees are an integral factor in agricultural and economic success with respect to their role in pollination, on top of their status as producers of beloved honey. Colony collapse disorder, a syndrome characterized by honey bees leaving the hive, is a detriment to Canada's $2.3 billion dollar honey bee-related agriculture industry, and the disorder is believed to be multi-faceted. Pathogens, weakening of honey bees due to pesticides, and environmental factors all may contribute to colony collapse disorder. Dr. Marta Guarna at UBC is part of a team located throughout Canada working on a proteomics initiative to elucidate a number of unknowns about colony collapse disorder and how to remedy them.

One aim of the project is to have a better understanding of pathogenesis of bacterial and viral infections that affect honey bees and the honey bee response to these pathogens. *Nosema ceranae* is a fungus under particular scrutiny, as its genetic material has been found in a statistically significant number of hives suffering colony loss. UBC Farm houses bee colonies for experiments and the lab also grows honey bee larvae *in vitro*. Dr. Guarna's lab works in close collaboration with beekeepers and bee breeders in British Columbia and Alberta, who are imperative parts of this project as it is meant to generate information that the beekeepers and bee breeders can use.

A second aim of the honey bee initiative is to identify markers of social immunity behaviours exhibited by individual bees within the hives. Much like humans have defenses against pathogens as part of our immune system, honey bees have behaviours that contribute to the success of the hive such as hygienic behaviour and *Varroa destructor*-sensitive hygiene. Hygienic behaviour can be described as recognizing diseased brood, uncapping it, and removing it to prevent the disease from disseminating into the hive. The *Varroa destructor*-hygiene is specific to behaviours that inhibit the reproduction of a fungal parasite that feeds on the hemolymph of the honey bee. Once markers of these social immunity behaviours are identified, then honey bee breeders may selectively breed the queen bees that have these markers to develop a fitter hive. This work is in collaboration with Dr. Steve Pernal of Beaverlodge Research Farm in northern Alberta.

10 years ago this project did not exist, as colony collapse disorder is a relatively new phenomenon that was first described, and subsequently garnered media attention, in the winter of 2006. The newly allocated funding for bee research correlated nicely with Dr. Leonard Foster's (UBC) long-term interest in honey bees and honey bee research; his family kept bees and Dr. Foster worked as an undergraduate in Dr. Mark Winston's laboratory at Simon Fraser University which studied honey bees. Dr. Guarna joined Dr. Foster at UBC's Centre for High Throughput Biology four years ago to work on the honey bee proteomics project.

Currently the researchers are in the process of narrowing down a panel of identified markers to be used in selective breeding to select queens that are better able to tolerate disease. The group has around 10 markers and is testing their efficiency as indicators of better hygienic behaviour in larger bee populations. A specific aim is to evaluate whether the panel can be refined to fewer markers and be equally indicative of the traits.

The project beginning included obstacles such as determining which samples yield enough protein to show correlation between expressed protein and traits related to disease-resistance. A variety of samples were assessed (antennae, midgut, hemolymph from larvae, and larvae tegument), but these samples were not equally predictive. For example, there was difficulty in obtaining enough protein from the larval hemolymph, but antennae provided substantially more protein associated with the hygienic behaviour traits. Although tissues other than antennae were rejected for the purpose of this project, the data on the honey bee midgut inspired a separate research project. Initially midgut was assessed due to its implications in bacterial infection, but there was no correlation to the field traits of interest. However, there was an interesting association between the proteins found in the midgut and the population of origin of the honey bees, and a project on the adaptations of honey bee populations to their environment has since been completed.

The project was truly made possible by the sequencing of the honey bee genome in 2004, and the goals of the project are continually shaped by working alongside the beekeepers and bee breeders of BC and Alberta, as listening to their needs helps shape the experimental plan for what is relevant to the industry. Multiple Reaction Monitoring (MRM), a technique for targeted proteomics, allows the researchers to verify proteins from a panel via multiplexed assays to quantify and analyze proteins in numerous honey bee antennae samples with greater sensitivity and specificity for the targets than a discovery (or shotgun) proteomics approach.

Once the panel is sufficiently refined, bee keepers will be able to send their tests to a specialized diagnostic lab for mass spectrometry assaying. Around two years from now, a test that will not require a specialized lab may be in development. This test likely will function as an ELISA to detect the panel of proteins within many samples with antibodies against the proteins linked to an enzyme. When the enzyme's substrate is added to the sample, a signal will develop as a colour change indicating presence of the protein. Possibly in the future a field test will be developed, which will allow honey bee keepers and breeders to identify bees with hygienic traits without sending the samples to a lab.

A future experiment within this project after testing for the healthier, more hygienic honey bees, which are associated with economic importance and overall fitness, is to give those producers selected queen bees and non-selected queen bees to directly assess the effect of MAS. Points of evaluation will include pollination, honey production, reproduction, disease management and economic output. This will indicate whether these protein markers are truly predictive of hygienic behaviour and whether they are traits amenable to selective breeding. Implications of this project are far-reaching. Identifying a small number of predictive markers that will facilitate marker-assisted selection (MAS) for breeding the most successful honey bee populations will increase understanding of the honey bee immune response and provide a way to transfer knowledge in a way that is helpful to beekeepers. Ultimately, beekeepers will have healthier bees, increased honey production, and sufficient pollination. And *that* is buzz-worthy.