**FINAL REPORT ON**

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**Research** Proposal

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## Objectives

1. Establish working sets of genetic markers for several key pollinators of blueberry
2. Quantify the basic population structure of different pollinators
3. Determine the relationships between genetic diversity of pollinator communities and different blueberry production systems

## Justification

There exists considerable variation in the social structures (social vs. solitary) and foraging behaviors of insect pollinators in blueberry. These species are important to seed- and fruit-set and may be negatively impacted—to differing degrees—through the effects of land-use practices (e.g., habitat fragmentation, mosaic landscape structure, and the application of broad-spectrum pesticides). However, with limited understanding of the population genetic structure of augmented- and native species, our understanding of the true impact of management practices on blueberry pollinator communities remains unknown. In the absence of such fundamental basic information, management strategies aimed at maximizing population connectivity and limiting the impact of pesticides on important pollinator species is purely speculative.

This proposal is a logical continuation of our previous studies on pollinator abudance, diversity, and efficiency in southeastern blueberries. Now armed with a detailed knowledge of the pollinator communities within these argroecosystems, we have taken this work to the next level and begun to elucidate the molecular ecology of this pollinator system to gauge its effects on blueberry production. Understanding the molecular ecology of native and augemented pollinators will enable us to (1) **determine the genetic impact of blueberry production practices on important polliantor groups**, (2) **determine the importance of genetic diversity for pollination efficency,** and (3) **make management recommendations to reduce any negative impacts due to production practices.**

## Methodologies

We identified and sampled from three blueberry fields, between 50 and 300 acres (a typical size range for North Carolina blueberry fields). Each site was visited at least three times during bloom, and multiple sites were visited each day. Pollinator species diversity appears to be a function of time and environment in southeastern blueberries, therefore multiple visit to a location were necessary to determine its average diversity. We visited sites on sunny, relatively calm (i.e., non-windy) days between 10:00 and 14:00 when bees were most active.

We collected a total of 53 individual bees. The honey bee (*Apis mellifera*), several species of bumble bees (*Bombus impatiens*, *B. citrinus*, *B. affinis*, and *B. bimaculatus*), the southeastern blueberry bee (Habrapoda laboriosa), two species of carpenter bees (*Xylocopa micans* and *X. virginica*), and many other "small natives" including *Osmia cornifrans*, *Osmia ligmaria*, *Halictus lubicundis*, *Lasioglossum* (Evylaeus), *Andrendi carlini*, *Halicuts parallelus*, *Lasioglossum* (Dialictus), *Andrena bradleyi*, *Ceratina calcarata*, and *Agaposemon splendas*. We also added another semi-managed commercial pollinator, the alfalfa leafcutter bee *Megachile rotundata*, to broaden the scope of the project to other model pollinator systems.

Collected bees were preserved in 95% ethanol during field collections for molecular analysis. All specimens were returned to Raleigh, NC, and stored at -80°C until processed. We then extracted whole genomic DNA from each individual using DNEasy® kits (Qiagen) and subjected them to standard PCR for analysis using molecular markers. We chose to employ microsatellite markers because of their high allelic diversity, cross amplification among species, and abundance in the genome. Because of the published sequence of the honey bee genome, we used eight loci of microsatellites each that have already been developed for *Apis* and *Xylocopa*, and we will soon screen another set of eight that have been developed for *Bombus*.

## Results

We screened eight *Apis* loci (A76, Ap43, Ap81, A113, A24, B124, A88, and ApJc2) and eight *Xylocopa* loci (XF24, XF26, XF42, XF3, XF38, XF31, XF20, and XF29) for all samples. We positively confirmed the amplification of the *Apis* and *Xylocopa* primer sets for their respective species, yielding high allelic diversity in each. More importantly, we were able to positively detect PCR amplification in other species. Specifically, we found cross amplification of Ap43 in the two *Xylocopa* species, A24 in *H. laboriosa*, and XF20

**Table 1**. Amplification of existing *Apis* and *Xylocopa* primers in several bee species. Successful amplification is indicated with a check, "nd" refers to non detectable amplification. These successes have enabled us to move forward on quantifying the population genetics of the pollinator community in blueberry.

| Type | Primer | *Apis mellifera* | *Bombus impatiens* | *Bombus citrinus* | *Bombus affinis* | *Bombus bimaculatus* | *Habrapoda laboriosa* | *Megachile rotundata* | *Xylocopa micans* | *Xylocopa virginica* |
|---|---|---|---|---|---|---|---|---|---|---|
| **Apis** | A76 | ✔ | nd | nd | nd | nd | nd | nd | nd | nd |
| | Ap43 | ✔ | nd | nd | nd | nd | nd | nd | ✔ | ✔ |
| | Ap81 | ✔ | nd | nd | nd | nd | nd | nd | nd | nd |
| | A113 | ✔ | nd | nd | nd | nd | nd | nd | nd | nd |
| | A24 | ✔ | nd | nd | nd | nd | ✔ | nd | ✔ | ✔ |
| | B124 | ✔ | nd | nd | nd | nd | nd | nd | nd | nd |
| | A88 | ✔ | nd | nd | nd | nd | nd | nd | nd | nd |
| | ApJc2 | ✔ | nd | nd | nd | nd | nd | nd | nd | nd |
| **Xylocopa** | XF24 | nd | nd | nd | nd | nd | nd | nd | ✔ | ✔ |
| | XF26 | nd | nd | nd | nd | nd | nd | nd | ✔ | ✔ |
| | XF42 | nd | nd | nd | nd | nd | nd | nd | ✔ | ✔ |
| | XF3 | nd | nd | nd | nd | nd | nd | nd | ✔ | ✔ |
| | XF38 | nd | nd | nd | nd | nd | nd | nd | ✔ | ✔ |
| | XF31 | nd | nd | nd | nd | nd | nd | nd | ✔ | ✔ |
| | XF20 | nd | nd | nd | ✔ | nd | nd | ✔ | ✔ | ✔ |
| | XF29 | ✔ | nd | nd | nd | ✔ | nd | ✔ | ✔ | ✔ |

and XF29 in one of the bumble bee species (*Bombus affinis* and *B. bimaculatus*, respectively) and the alfalfa leafcutter bee (Table 1). The amplifications yielded significant PCR product for each, thus there will serve as robust molecular markers for the purposes of population genetic analyses.

## Conclusions

We successfully accomplished our first objective, which was to develop working sets of genetic markers for several key pollinators of blueberry. In addition to the known *Apis* and *Xylocopa* marker sets, and a forthcoming *Bombus* set of eight microsatellites, we were also able to cross-amplify at least four microsatellite loci in four other species. This will enable us to make relative comparisons of allelic- and genetic diversity across pollinator types, as well as to quantify overall population genetic diversity. We are now focusing screening efforts on native bee species, specifically *Andrena bradleyi,* the most

common native bee in blueberry fields after bumble bees and the southeastern blueberry bee (*Habropoda laboriosa*). With the developed marker sets from this project, and with additional sampling effort both in the field and from previously collected samples preserved in the NC State University Insect Museum, we will be able to quantify the basic population structure of different pollinators and determine the relationships between genetic diversity of pollinator communities and different blueberry production systems (Objectives 2 & 3). This will be the focus of our activities in winter 2013.

## Impact Statement

We have identified several primer sets that can be used for native bees common in the southeastern blueberry system, including the first primer confirmed to work for *H. laboriosa*, a specialist native blueberry pollinator. This gives us the ability to compare bee genetic diversity between production practices in blueberry.

## Citation(s) for any publications arising from the project

None at this time. We will soon be submitting a grant proposal to the Foundational Program of the USDA-AFRI (February 2013). The results generated by this project will greatly enhance the likelihood of its favorable reception by the panel.