Transgenic injection in the western honey bee (*Apis mellifera*)

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IGTRCN P2P Training Fellowship Report

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Objectives of the Visit

Background

The western honey bee (*Apis mellifera*), has vital ecological and economic importance as a food producer and pollinator. It is also a model system for social behavior research (Zayed and Robinson, 2012). Honey bees live in complex societies (colonies). They are eusocial animals characterized by several distinctive features: overlapping generations, cooperation of brood care and division of labor. There is division of labor in honey bee reproduction: queens reproduce while workers perform all the rest tasks. There is also age-dependent behavior shift in workers. Individual workers change their tasks multiple times in their lives. There are also advanced perception, complicated communication and coordination between bees. Thus, it provides a great platform to study and understand the neurobiological, genetic and genomic mechanisms behind their social behavior.

To make bees accessible to genetic manipulation, effective methods of gene knock-down or ectopic expression are needed. RNA interference (RNAi) technique has been devised to knock down gene expression in honey bees. Researchers used either double stranded RNA(dsRNA) targeting long stretches in the gene of interest (Ament et al., 2012; Beye et al., 2002; Nelson et al., 2007) or much shorter small interfering RNAs(siRNA) (Li-Byarlay et al., 2013). Multiple delivery options are also available from traditional embryonic injection(Beye et al., 2002), abdominal injection(Nelson et al., 2007), feeding(Aronstein et al., 2006) to the creative nebulization (Li-Byarlay et al., 2013). Although the progress in RNAi has already led to fruitful discoveries, robust transgene technique is an essential step in establishing the western honey bee as a model organism. It will enable us to overexpress genes of interest, which is impossible with RNAi. In the past, there were several transgenic attempts with *Apis mellifera* and eastern honey bee species *Apis cerana* (Guo et al., 2007; Robinson et al., 2000), however, no events of stable genomic integration were observed. Martin Beye's lab made the technology breakthrough by using the *piggyBac* transposon to generate stable transgenic honeybees at high efficiency (Schulte et al., 2014), which opens many new possibilities for honey bee researchers (Ben-Shahar, 2014).

The priority goal of the Robinson lab is to study the genetic and genomic mechanisms governing western honeybee's social behavior. Genomic and RNAi experiments in have identified key master regulators in brain that control worker bee behavior maturation and division of labor (Ament et al., 2012; Chandrasekaran et al., 2011). Currently the genetic manipulation method in our lab is limited to RNAi knock down of gene expression.

Trip purpose

The purpose of my short trip to the Beye Lab was to learn and bring honeybee transgene technique to our toolbox of social behavior research in the Robinson lab.

Accomplishments and the degree of success

In the fourteen day trip, I learned the detailed experimental set up for honeybee embryonic injection. Since the duration of trip was too short for a round of complete transgenic experiment, I focused on hands-on practice of the embryo collection and transgenic injection (Fig1A-C, E and F). To learn the remaining bee rearing of the protocol, I talked extensively to Beye lab members and visited their facility (Fig 1.D).

I practiced egg collection (Fig 1A) and preparation for injection (Fig 1B). The injections were done under the microscope with pico-injector and micromanipulator (Fig 1C).
To control whether I can deliver DNA into embryos, I brought an expression plasmid with GFP under the control of *ubiquitin* promoter. I used green fluorescence as a marker to test if my injections were able to successfully deliver DNA into embryos. Green fluorescence were observed in the larvae survived embryonic injection. The signal was usually in the posterior and central part, where the embryos were usually penetrated.

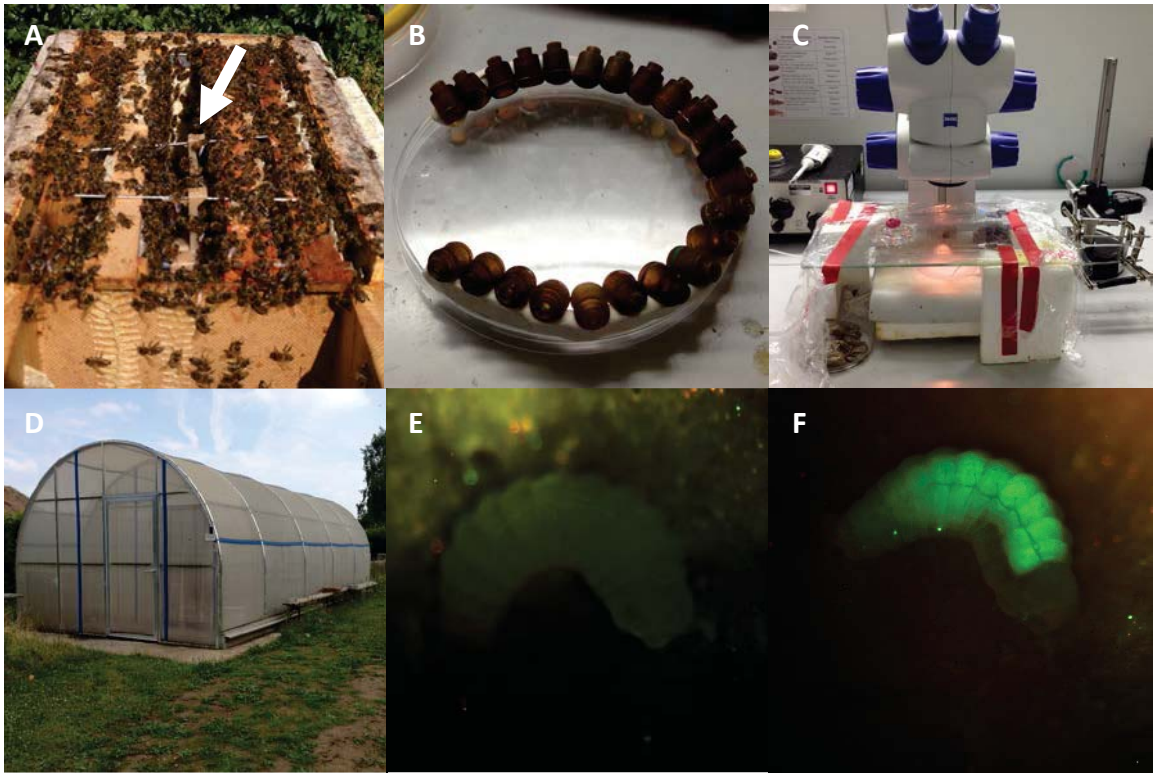

Figure 1. Learning injection setup in the Beye Lab. (A) Honeybee queen caged in a Jenter box (arrow) positioned between frames. In the Jenter box, the queen laid eggs in small plastic cups. B) Honeybee embryos in small plastic cups were prepared for injection. A ring of playdough attached the plastic cups to a Petri dish. (C) Experimental setup for embryonic injection. Embryos were injected under the microscope in the humid chamber made from plastic panels and Styrofoam blocks. A pico-injection machine (PLI-100, left in the picture) and injection needle mounted on Oxford micromanipulator (right in the picture) delivered DNA to embryos. (D) The Beye Lab keeps transgenic honey bee lines in a fully enclosed flight house. (E) Control embryos injected with water under UV fluorescent microscope. (F)Embryos injected with Ubi>GFP plasmid under UV fluorescent microscope. Green fluorescent was oberved.

Acknowledgements

I am greatly grateful to Dr. Gene Robinson and Dr. Martin Beye for making my visit possible. I am thankful to the Beye lab for the great training experiences with honey bee transgenic technique: Dr. Christina Vleurinck for general protocol explanation, embryonic injection training and microscope imaging assistance; Max Ewinger for queen caging, egg collection training and knowledge about post injection rearing; Dr. Marianne Otte for knowledge about transgenic bee line maintenance.

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