# REGULATION OF HONEY BEE APPETITE INDEPENDENT OF THE GLUCOSE INSULIN SIGNALING PATHWAY

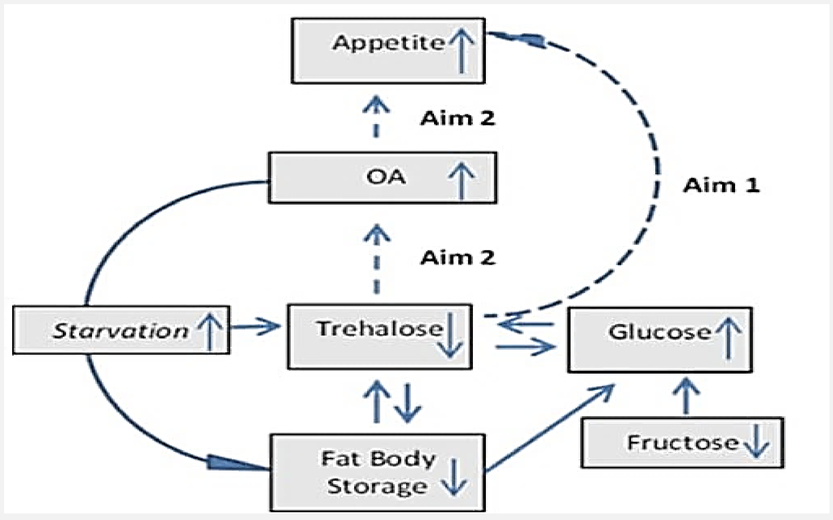

- STUDENTS / UNIVERSITIES
  Elif Filiztekin/ Sabancı University
  Furkan Yaşaryıldız/ Istanbul University
  Faruk Yıldıztekin/ Bezmialem Vakıf University
- SUPERVISOR(S)
  Asst. Prof. Christopher Mayack


Sabancı Üniversitesi

PURE PROGRAM FOR UNDERGRADUATE RESEARCH


## ABSTRACT

Declining pollinator (bee) health is now a global concern. The honeybee plays an economically important role with its pollination of agricultural crops being valued at $15 billion in the US and 22 billion EURO in the EU each year.[1] The maintenance of energetic homeostasis and appetite regulation are both well-studied areas,[2] but how the two are connected mechanistically, the metabolic pathways linking appetite and the energic state, remain largely unknown. Our aim is to map the relationship between energetic stress, physiological response, and behavior. A behavioral assay, the Proboscis Extension Response (PER) to measure appetite,[3] was integrated with well-controlled physiology experiments, which involve using minimally invasive localized injections to determine the mechanisms underlying energetic state and appetite regulation that are independent from the glucose insulin signaling pathway. We found that particular sugars cause different appetite responses and this was dependent upon the age class of the honey bee.


***Schematic Overview of Research Aims:*** How regulation of sugar levels may be connected to octopamine (OA) and the regulation of appetite. Solid lined arrows are already established connections, while dotted arrows are hypothesized connections. The arrows to the right of each label represent predicted modulation changes expected due to starvation.

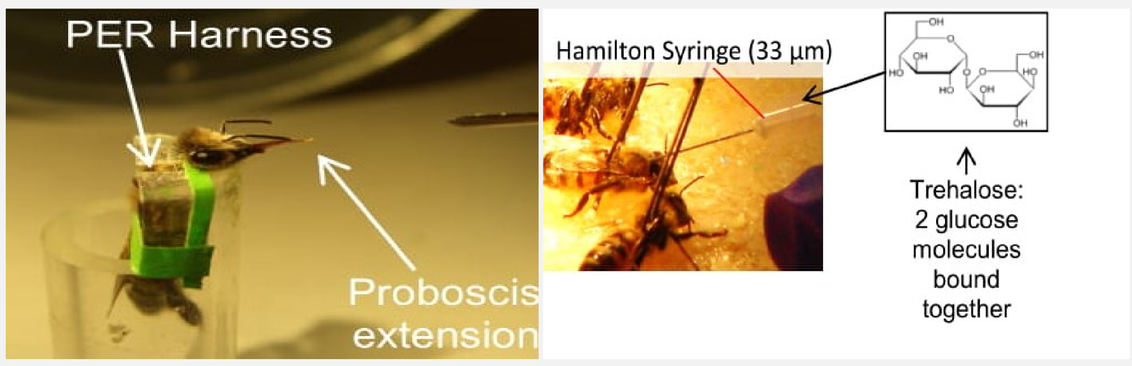
## OBJECTIVES

***Aim 1***: Determine the relationship between fluctuating sugar levels and appetite regulation.

***Aim 2***: Determine if octopamine (OA) and which of its receptors is the link between fluctuating trehalose levels and appetite.

***Aim 3***: Establish that age – related OA baseline levels in the brain is responsible for the variation in appetite regulation as the bee ages.

## PROJECT DETAILS




**Immobilization:** Bees from each group were collected in glass vials (N = 40 at a time) and immediately placed on ice to be immobilized.

**Harnessing:** Immobilized bees were put in straws and banded with sticky black tapes in a way that the thorax of bees' is open and easily injectable with Hamilton syringe.

**Injection:** Each group was injected with 1 µl of 1.5 M of trehalose plus trehalozin. For the monosaccharides glucose, fucose, and fructose a concentration of 3 M was injected to match the w/v % equivalent concentration of the trehalose injection. Each sugar was injected into the hemolymph through the thorax using a Hamilton microsyringe.

**PER Assay:** This process involves touching the bees antennae with a series of sucrose solutions (0, 0.1, 0.3, 1, 3, 10, and 30%) in ascending order of concentration.

**Hemolymph Extraction:** Following the PER assay, 1 µl of hemolymph was extracted using a glass microcapillary. (Sigma Aldrich)

**Storing**: After the extraction, hemolymph was stored at – 80° C until sugar level analysis with Gas Chromatography Mass Spectrometry (GC MS) can be completed.

## Results

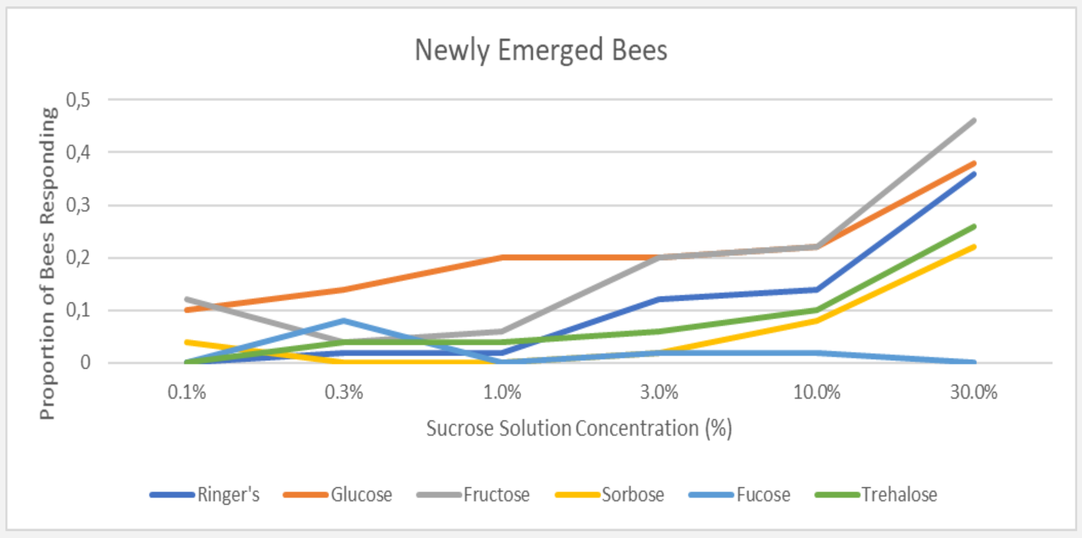



***Figure 1: The newly emerged bees*** display varying levels of response based on sugar type, except for the Ringer's-injected bees which act as a reference. While responses of glucose-, fructose-, and trehalose-fed bees are as expected, sorbose- and fucose-fed bees diverge from the hypothesis.

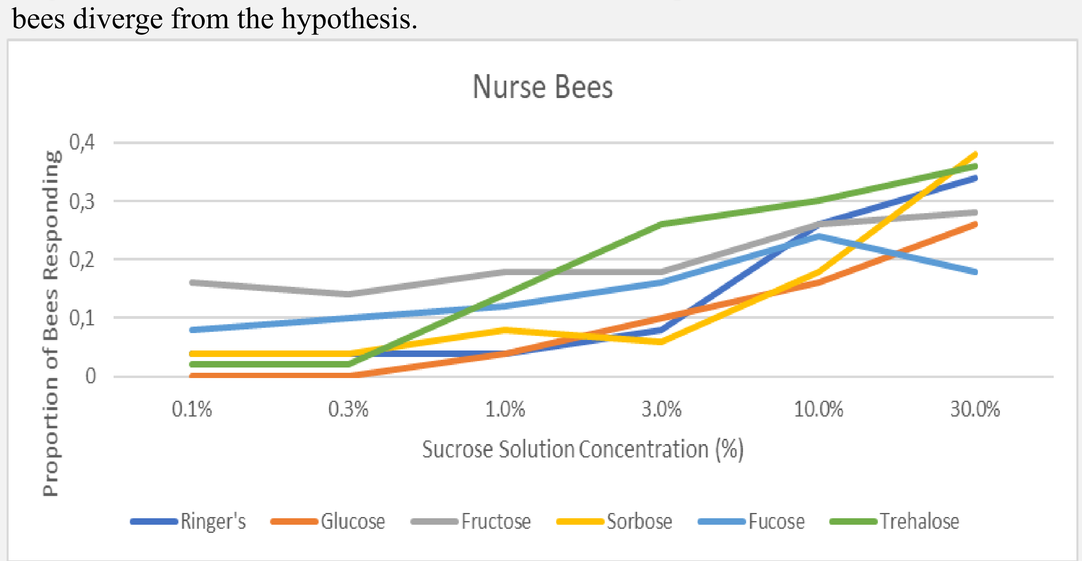



***Figure 2: For the nurse bees***, fructose, fucose, glucose, and sorbose groups display expected response levels; whereas the trehalose group – from which the lowest response was expected – demonstrated the greatest appetite.

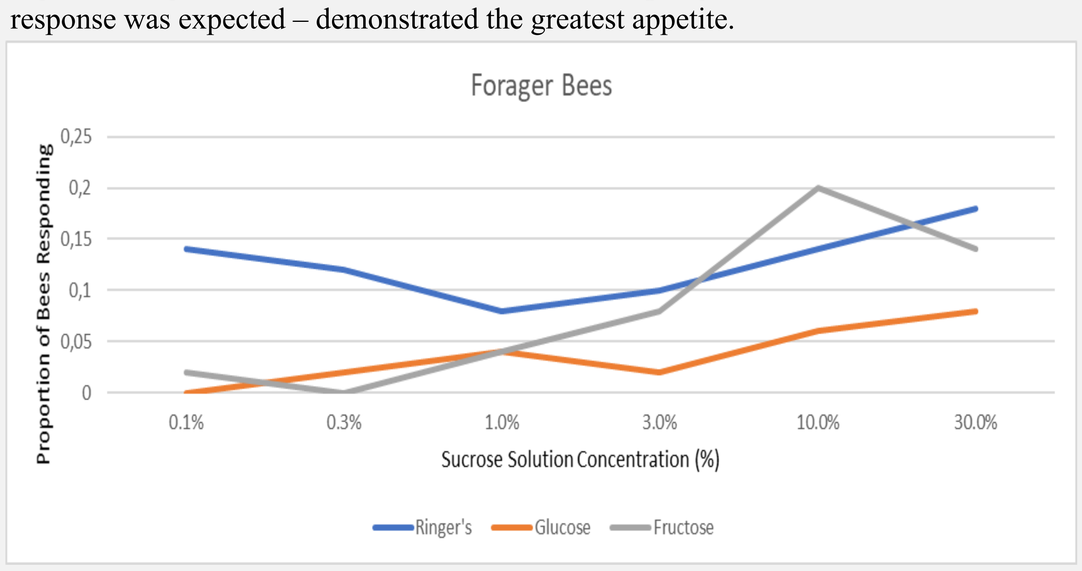



***Figure 3: For the forager bees***, the data collected so far seems to indicate that glucose suppresses the appetite, whereas fructose provides a more variable response.

## DISCUSSION AND FUTURE WORK

The responses of newly emerged and nurse bees seem to vary a lot both within and between age groups. Although there are more groups to be done for foragers and statistical analysis is yet to be done, foragers seem to display more stable responses; which may be due to the required specificity of energy during foraging. The steps to further analyse the relationship between hemolymph-sugar levels and appetite are:

**1)** State-of-the-art molecular RNAi and CRISPR cas-9 techniques to determine the mechanisms underlying energetic state and appetite regulation will be included.

**2)** Long term goal of this project is to understand the neural basis between fluctuating blood sugar levels and appetite regulation as the bee ages, so we can understand how the two can become uncoupled with a parasite infection.

## REFERENCES


**1.** Southwick, E. E. & Southwick Jr, L. 1992. "Estimating the Economic Value of Honey Bees (Hymenoptera: Apidae) as Agricultural Pollinators in the United States", Journal of Economic Entomology 85 (3), 621-633

**2.** Pankiw, T., Waddington, K. & Page, R. 2001. "Modulation of sucrose response thresholds in honey bees (Apis mellifera L.): influence of genotype, feeding, and foraging experience", Journal of Comparative PhysiologyA: Neuroethology, Sensory, Neural, and Behavioral Physiology, 187 (4), 293-301.

**3.** Wang, Y., Brent, C. S., Fennern, E. & Amdam, G. V. 2012. "Gustatory Perception and Fat Body Energy MetabolismAre Jointly Affected by Vitellogenin and Juvenile Hormone in Honey Bees", PLoS Genet, 8 (6) e1002779.