TRANSGENERATIONAL IMMUNE PRIMING

How insects pass immunity to their offspring

Photo by Amy Floyd

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Dalan ANIMAL HEALTH

Introduction to Insect Immunity

The Challenge of Disease

All organisms are constantly faced with the challenge of disease. Animals, including insects, and plants have all adapted unique ways to protect themselves from infections. Insects have developed both social and physiological immune mechanisms to protect themselves against threatening pathogens, such as removing themselves from a colony when sick, mummifying pests that enter their home, and phagocytizing foreign invaders. While they do not possess as sophisticated immune systems as mammals, insects, such as bees, exhibit robust immune processes that protect them from disease.

Like all other animals, insects have an innate, unspecific, immune system to respond to pathogens. For example, during an infection, they secrete antimicrobial proteins or form large aggregates that act as a protective barrier. Protection can be long lasting and is sometimes specific to the pathogen already encountered, but the specificity of the protection remains unknown. Unlike vertebrates, insects do not have antibodies that allow them to recognize pathogen antigens that they have previously encountered.

Maternal Transfer

One example of immune priming in vertebrates is the mechanism behind transfer of maternal immunity to their offspring. Females pass immunity to their offspring by providing hormones, nutrients, as well as protective antibodies. These immunity building factors are transferred to the offspring via the mother's placenta, egg yolk, or milk during lactation. As a result, the progeny has a basic protection to disease while develop their own specific immunity.

It was previously thought that insects do not possess any kind of long lasting immunity. However, within the last decade, scientists have discovered more complexities to insect immunity, which include some features typical for acquired immune system. Furthermore, research has shown that insects too can pass information from one generation to the next, through a mechanism called Transgenerational Immune Priming, or TGIP. This mechanism appears to be highly conserved across invertebrates, as it has been observed and studied in 25 different species so far. In this white paper, will focus on the research done with the honeybee Apis mellifera.

First Demonstration of TGIP in Honeybees

The first demonstration of TGIP in honeybees was published in 2014 by Hernández López, et. al. In this study, honeybee queens were injected with dead Paenibacillus larvae, a spore-forming gram-positive bacteria that infects honeybees, and dead E. coli, a gram-negative bacteria. The subsequent offspring of the pathogen-injected queens were challenged with spores of both P. larvae and E. coli. and mortality rates were measured. Offspring from primed queens were 26% more likely to survive when faced with the pathogens than controls. The researchers concluded that immune priming by the maternal insect protected the offspring and increased survival. What was still to be answered was the mechanism behind this immune transfer, since this mechanism must be mediated by something other than antibodies, which insects do not possess.

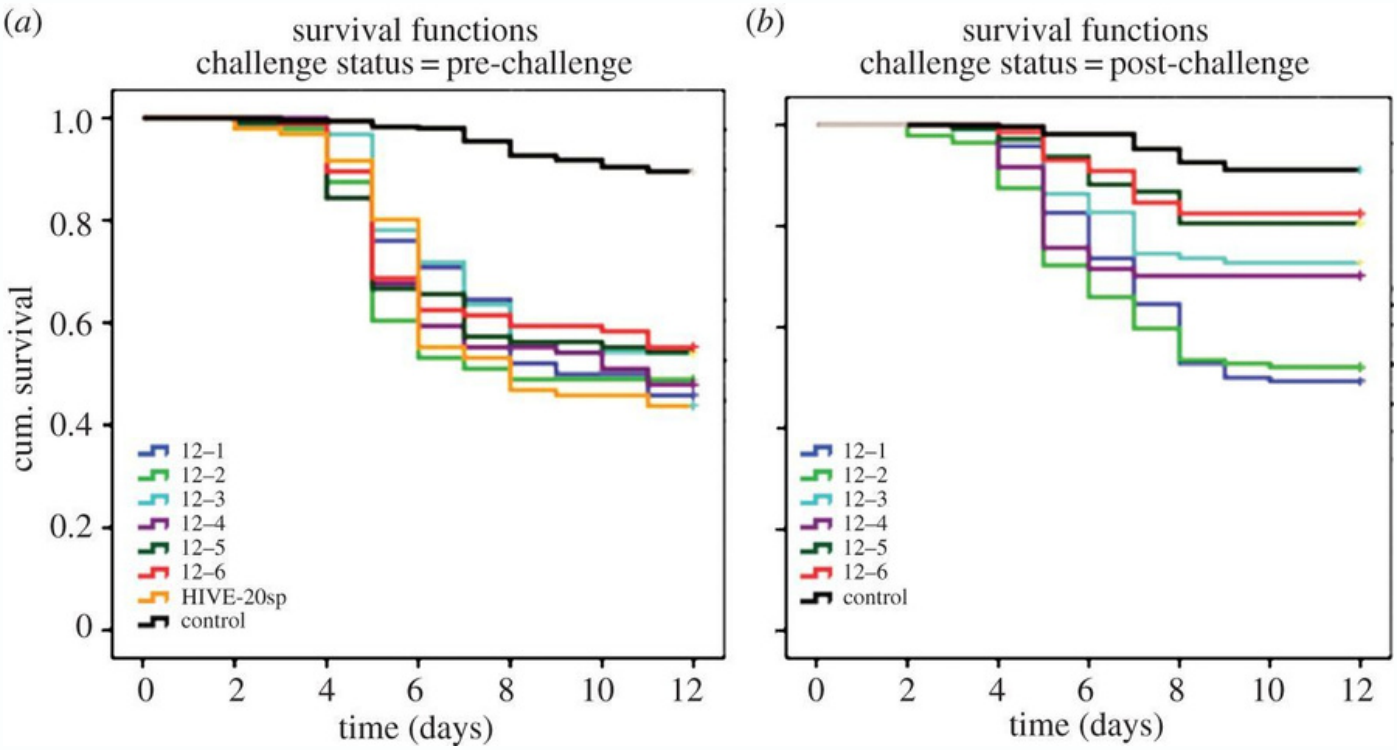
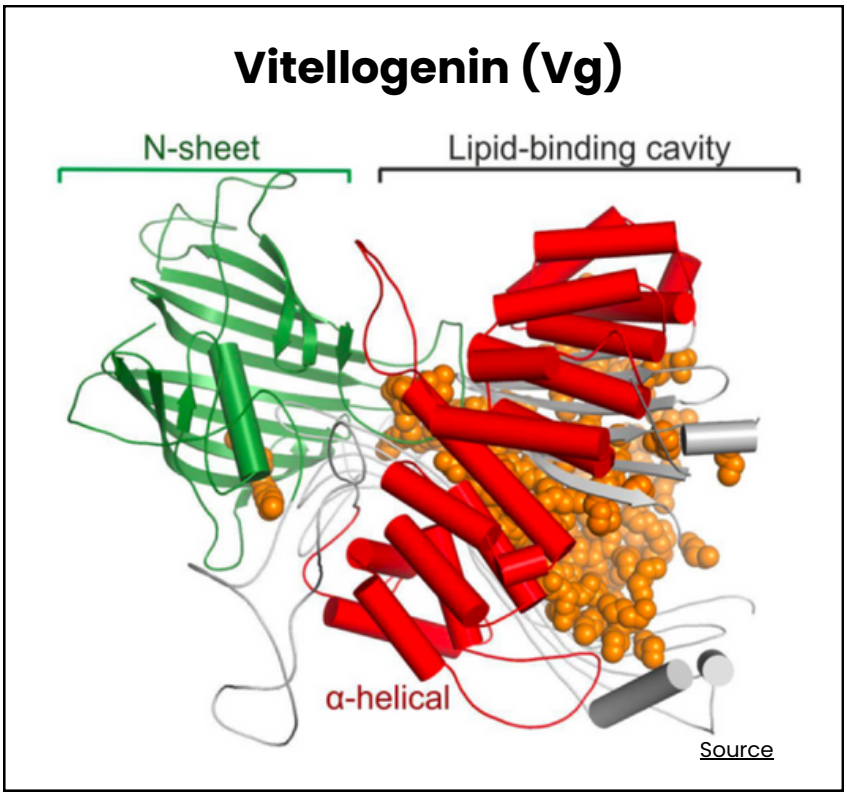


Figure 1: Survival profile of larvae of nucleus colonies exposed to 20 spores of PI, pre-challenge (a) and post-challenge (b).

Establishing the Essential Protein

In 2015, Salmela and Freitak, two researchers at the University of Helsinki in Finland, in collaboration with Gro Amdam, published a key mechanism underlying TGIP in the Journal of Insect Physiology. The research established that the egg-yolk protein Vitellogenin (Vg), an essential egg-yolk protein and distributed widely in egg-laying animals, was an essential carrier of immune elicitors to the next generation of honeybees. Using immunofluorescence microscopy and western blotting, the authors showed that vitellogenin bound to bacteria fragments, both P. larvae, the gram-positive bacterium causing American Foulbrood disease, and E. coli, a gram-negative bacterium.



By fluorescing tissue sections of queen honeybee eggs, the authors determined that Vg was required for transporting pieces of bacteria into the eggs. This paper identified vitellogenin as the carrier of immune-priming signals. This work reveals a molecular explanation for trans-generational immunity in insects and a previously undescribed role for vitellogenin.

This study identified Vg as the carrier of immune-priming signals and revealed a key molecular explanation for TGIP in honeybees. By establishing a molecular pathway for immune priming, the door opened to envision novel ways to help protect honeybees from diseases, such as vaccination.

TGIP at a Colony Wide Level

How can these findings be applied to honeybees in their social environment?

The question remained on how a honeybee queen would encounter a pathogen to pass on the information to her offspring. Honeybees are social insects. The honeybee queens rely on thousands of worker bees to build and maintain the colony, rear brood, forage for nectar and pollen, and feed her by producing a nutritional substance called royal jelly. Therefore, it is unlikely a queen honeybee would encounter a pathogen on her own.

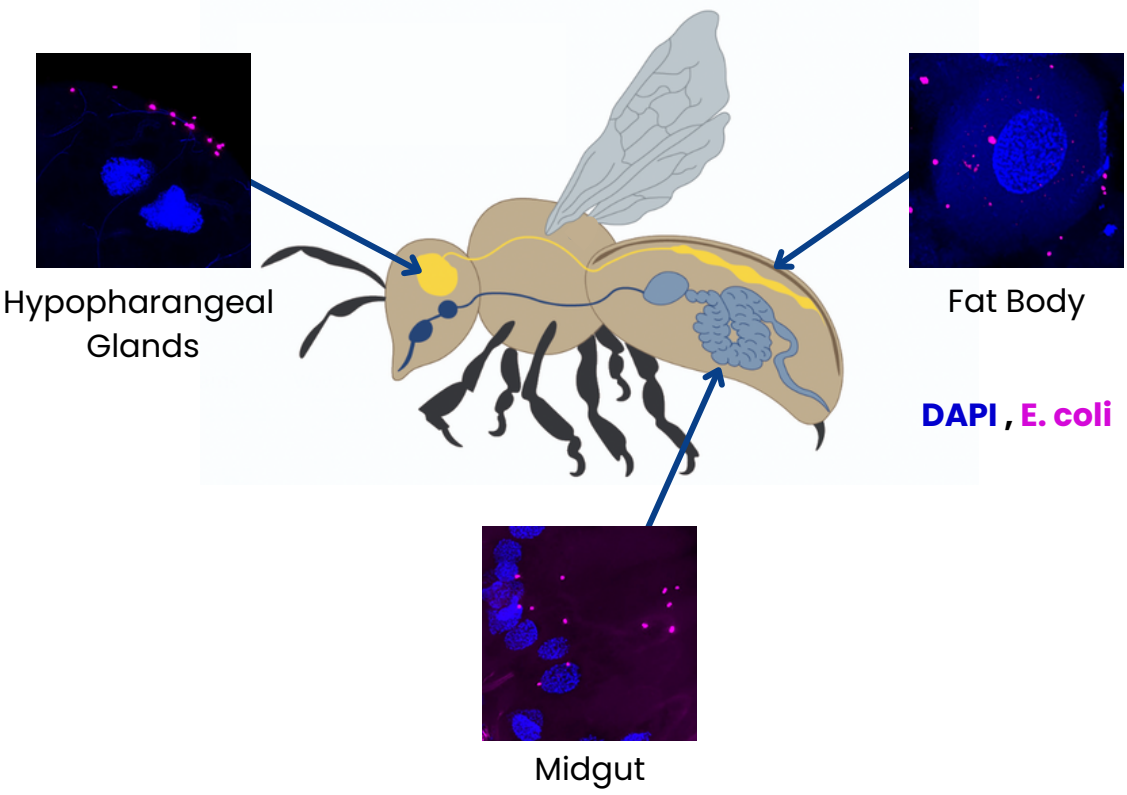
In 2019, Freitak collaborated with Gyan Hardwood and Gro Amdam at Arizona State University to reveal the critical interplay between the worker bees and the honeybee queen in TGIP. Utilizing immunofluorescence microscopy, qPCR, and RNAi gene knockdown experiments, the study demonstrated that Vg bound to bacterial fragments that was ingested by worker bees. The fragments were transported to the hypopharyngeal glands, the site of royal jelly production in the worker bees. Workers naturally feed the pathogen containing royal jelly to the honey queen bee. These results provide initial evidence that TGIP may operate at a colony wide level in honeybees.

This study did not conclude whether bacterial fragments are secreted directly into the royal jelly, or if they only bind to the gland surface to illicit an immune response by the glands. In 2021, Harwood et. al. determined that when bacteria is ingested by a worker bee, pathogen fragments are directly incorporated into the royal jelly. This ingestion also induces higher levels of defensin-1, an anti-microbial peptide, in the royal jelly. Studies have shown honeybee larvae challenged with American Foulbrood illicit little to no expression of defensin-1, making them more suseptible to the infection. Therefore, additional defensin-1 supplied by nurse bees may also aid in fighting infection.

Summary of TGIP in Honeybees

To summarize, these studies identified the mechanism by which immune priming can exist at a colony wide level. When worker bees ingest a pathogen, it is digested and transported to the midgut, where they are lysed. The bacterial fragments are transported through the epithelial lining of the gut into the fat body, which is an intermediate for any transfer in and out of the gut and the site of vitellogenin synthesis. Once in the fat body, the bacterial fragments bind to protein vitellogenin. The Vg-bound pathogen is then absorbed into the hemolymph, the circulatory system of insects, where it is transferred to the hypopharyngeal glands. The bacterial fragments are directly incorporated into the royal jelly.

Fluorescence of E. coli fed workers (Hardwood et. al., 2019)



It is hypothesized that once the honeybee queen ingests the royal jelly, the bacterial fragments make their way from the midgut to the hemolymph following the same process above. In the hemolymph, the Vg bound bacterial fragments are transported into the ovaries where the developing eggs are housed. This route would allow the queen to prime developing offspring with the target pathogen. The subsequent immune primed eggs will develop as larvae resistant to infection of the pathogen.

The World's First Approved Honeybee Vaccine

A new solution

The work on the underlying mechanism behind TGIP on the individual level and growing knowledge of immunity at the colony level helped lay the groundwork for the development of the first ever insect vaccine. This has allowed us to develop an industry-first method to impart protection and improve resilience to brood disease.

How it works

The Dalan vaccine technology exposes queen bees to inactive (dead) bacteria, which enables the larvae hatched in the hive to resist infection.

Questions? Contact us at 1-844-483-2526 or www.dalan.com/contact