2023

# LINKING PREVIOUS EXPERIENCES TO BEHAVIOR AND HEALTH IN THE HONEY BEE (*Apis mellifera*)

Rebecca R. Westwick
*University of Kentucky*, r.r.westwick@gmail.com
Author ORCID Identifier:
https://orcid.org/0000-0001-7871-9988
Digital Object Identifier: https://doi.org/10.13023/etd.2023.412

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Rebecca R. Westwick, Student

Dr. Clare C. Rittschof, Major Professor

Dr. Charles W. Fox, Director of Graduate Studies

# LINKING PREVIOUS EXPERIENCES TO BEHAVIOR AND HEALTH IN THE HONEY BEE (*Apis mellifera*)

---

DISSERTATION

---

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture, Food, and Environment
at the University of Kentucky

By
Rebecca R. Westwick
Lexington, Kentucky
Director: Dr. Clare C. Rittschof, Professor of Entomology
Lexington, Kentucky
2023


https://orcid.org/0000-0001-7871-9988

ABSTRACT OF DISSERTATION

LINKING PREVIOUS EXPERIENCES TO BEHAVIOR AND HEALTH IN THE HONEY BEE (*Apis mellifera*)

An organism's ability to respond to changing conditions can be vital to its success. Indeed, plasticity is a common feature of living organisms. Much of the research in this area, though, has focused on effects caused by environmental conditions. What has received relatively less attention is how social experiences and broader features of an organism's social environment can lead to long-lasting changes in health and behavior. This knowledge gap exists despite the well-documented existence of health and behavioral effects after social interactions in certain taxa such as humans.

Social insects such as honey bees provide an excellent opportunity to better understand this phenomenon due to their well-characterized behavioral repertoire, complex social dynamics, and experimental tractability in natural and semi-natural settings. This project examines multiple aspects of honey bee behavior and health to determine how they are affected by a bee's previous experiences. Additionally, this project aims to uncover how elements of the social environment (such as colony-level aggression) lead to different outcomes in adult behavior, physiology, and health in these insects.

I first documented the existence of high colony-level variation in the nutritional profile of "worker jelly." Worker jelly is a nutritional secretion that is synthesized by adult nurse bees and comprises the entirety of the nutritional resources available to a honey bee larva, making it a critical feature of the early-life development period for bees. Next, I examined the social interaction element of nurse bees inspecting and feeding larvae. I determined that this vital interaction can be affected by social pheromones such as the honey bee alarm pheromone. This effect was dependent on the colony-level aggressive social environment, however, despite these nurses not being specialized for aggressive nest defense. I then followed up on the previous results by using electrophysiology to determine that colony-level aggression differentially affects the peripheral detection of some social pheromones in nurses but not in bees of a typically more aggressive task specialization, foragers. Finally, I turned the lens to the adult social interaction of allogrooming. Allogrooming is a key component of a honey bee colony's health-promoting "social immunity." I tested how an acute allogrooming event affects the expression of key immune genes from multiple pathways as well as deeply conserved genes implicated in social responsiveness across taxa.

This work demonstrates how early life experiences and social interactions can affect the health and behavior of a highly social organism. Additionally, given the recent challenges faced by these important pollinators, this research provides key foundational knowledge on the importance of social factors in maintaining the overall health and vitality of honey bees and honey bee colonies.

KEYWORDS: honey bee, developmental behavioral plasticity, early life nutrition, peripheral sensory sensitivity, parental care, allogrooming

Rebecca R. Westwick

*(Name of Student)*

08/10/2023

Date

LINKING PREVIOUS EXPERIENCES TO BEHAVIOR AND HEALTH IN THE HONEY BEE (*Apis mellifera*)

By
Rebecca R. Westwick

Dr. Clare C. Rittschof
Director of Dissertation

Dr. Charles W. Fox
Director of Graduate Studies

08/10/2023
Date

ACKNOWLEDGMENTS

I would like to extend thanks to the many people and entities who supported me during the creation of this work.

First and foremost, to my amazing advisor—Dr. Clare Rittschof—for her invaluable mentorship and guidance.

To the members of my committee—Drs. Kenneth Haynes, Charles Fox, Jessica Santollo, and Zainulabeuddin Syed—for their leadership and helpful feedback on this document and everything else to prepare me for being a scientist.

To all members of the Rittschof lab—Grayson Grume, Joseph Palmer, Amanda Denny, Jimmy Harrison, Caroline Kane, Anastasia Weger, Wade Pike, Drs. Allison Young, Erin Treanore, and Chelsea Pretz—for their unwavering camaraderie through the sweat and the stings.

To all of the undergraduate researchers who assisted with field work and data collection for my projects—Anna Foose, Gavin Brackett, Bethany Ison, Cameron Brown, Taylor Napier, Abdallah Sher, Molly Carson, and Sophia Cabrera—for their hard work and dedication to science.

To the entities that funded me—the University of Kentucky, the Animal Behavior Society, the National Science Foundation, and the Sigma Xi Society—for affording me the resources necessary to complete this work.

To the many bees who participated in my experiments, without whom there would be no data.

To the instructors of my courses, for the wisdom and knowledge they imparted.

To the things that kept me Human—my Saoirse/Astra friends, the White Ash group, the Fu Crew, Woodland Christian Church, and every friend who checked in on me during the course of my PhD (Cece, Shannon, Tracy, Cetta & Wayne, Valerie, and Dennis & Nikita among others). Additionally, to the entertainers that kept me sane during many long hours in the lab—Dimension 20, Otzdarva, OhTofu, DBDLeague, the Houston Astros Radio Broadcast team (especially Robert Ford and Steve Sparks), Meg Turney, How To Cook That, and many others.

To my wonderful pets—Bonny, Tonks, Perrin, and Sunny—for giving me a reason to get up from my computer and get some fresh air every day.

To my family—both blood and in-law, too numerous to list here—for your unconditional love and enthusiastic cheerleading. You all mean the world to me, and I am so lucky to call you family.

And finally, to my amazing husband—Dr. Andrew Sickmeier—without whom I wouldn't have made it in one piece. For his gracious patience when I needed takeout for dinner, for never complaining when my schedule got weird, and for always having a warm hug when I needed it.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# BACKGROUND INFORMATION

Behavior and health are strongly interrelated in animals. For example, poor health can alter behavior. Many animals will display "sickness behaviors" when experiencing an immune challenge, with symptoms such as social withdrawal and changes to sleep, appetite, and attention (Tizard, 2008). Behavioral changes are consequently one of the most valuable non-invasive tools for detecting health changes in animals that are under human observation (Weary et al., 2009). Conversely, behaviors can feed back into an organism's health. For instance, social interactions have been demonstrated to have major consequences for the gut microbiome across a wide range of taxa, and social touch is associated with a range of positive immune and health outcomes (Ang et al., 2012; Cohen et al., 2015; Ezenwa et al., 2012). Behavior and health therefore form complex feedback loops, mutually affecting one another yet also individually subject to outside forces.

Behavior, like many other phenotypes, can change throughout an individual's lifetime based on its experiences and environment (Hobert, 2003). This concept is known as "plasticity" (West-Eberhard, 2003). For example, male birds can plastically change the frequency of their calls based on the current environmental noise levels (Gross et al., 2010). This ability to respond to current conditions can be critical for survival and reproduction (Flynn & Smee, 2010; Han & Brooks, 2014). One of the strongest and most common scenarios where we see effects of plasticity is during early life, which is referred to as "developmental plasticity" (Beldade et al., 2011). Developmental plasticity allows organisms that are already in a period of growth and change to target their adult phenotype to the current environmental optimum (Nettle & Bateson, 2015). On the other hand, though, developmental plasticity often leads to lasting changes, which means that deficits acquired

during this period often cannot be fully compensated for later if conditions change (Hoverman & Relyea, 2007). Common manifestations of this phenomenon can be seen with cases such as critical periods for behavioral development or irreversible physiological changes due to early-life nutritional conditions (Alippi et al., 2002; Beach & Jaynes, 1954; Hensch, 2004; Scott, 1962). Empirical work into plasticity in general—but developmental plasticity in particular—has focused more heavily on the effects of the environment at the expense of research into the effects of social inputs, particularly for social effects that are persistent (Han & Brooks, 2014; Kasumovic & Brooks, 2011). Combined with the aforementioned links between behavior and health, there is a clear need to better understand how previous experiences affect behavior and health in the context of sociality.

The western honey bee (*Apis mellifera*) serves as an excellent model system to study how previous experiences alter behavior and health. Honey bees demonstrate a range of complex behaviors, and their degree of sociality rivals that of humans (Seeley, 1989). They are amenable to being studied in controlled laboratory experiments. There are many well-established paradigms for honey bees, such as small dish assays to measure behavior at an individual scale or tests for associative learning with the proboscis extension reflex (PER) of harnessed bees (Marfaing et al., 1989; Richard et al., 2008). And yet they can also be studied in more naturally and ecologically relevant contexts as well, which can be a difficult translation for many of the more traditional lab-based model systems (Yartsev, 2017). For example, colony- and even population-scale experiments are common in these insects, as are transitional scales such as observation hives that allow for detailed examination of a functional colony in a nearly natural setting (Bortolotti et al., 2003; Bozek et al., 2021; Brouwers et al., 1987; Couvillon & Ratnieks, 2015). Furthermore, many of the

laboratory tools that have been developed for model systems (e.g. CRISPR/Cas9) have been adapted for these widely studied insects (Kohno et al., 2016). The honey bee genome was one of the earliest insect genomes to be sequenced and was completed around the same time as chimpanzee genome (Waterson et al., 2005; Weinstock et al., 2006). Even some high-level neurobiological techniques such as in-vivo calcium imaging have been adapted for these insects (Joerges et al., 1997). Thus, honey bees provide ample opportunities to investigate both physiological processes and ecological outcomes for health and behavioral effects.

Another feature of honey bees that supports their use in research involves the complex social dynamics and behaviors that characterize these insects. A colony consists of one queen, who is the only reproductive female and the mother of all other bees in the colony (Page & Robinson, 1991). She will mate with multiple males on a "nuptial flight" shortly after she emerges as an adult, at which time she will return to the colony and remain there to lay eggs for the rest of her life (unless the colony "swarms," which is how new colonies are formed) (Koeniger, 1986). Honey bees have a haplodiploid reproductive system (Page & Robinson, 1991). If the queen lays an unfertilized egg, it will develop into a haploid male drone. Drones are primarily geared for reproduction; they have very limited roles in the day-to-day functioning of the colony and die immediately after mating (Ayup et al., 2021; Bonsels, 1912; Kovac et al., 2009). If the queen lays a fertilized egg, it will develop into a diploid female. The vast majority of these females will become sterile workers. A small percentage may develop into new, reproductively capable queens (Page & Robinson, 1991). The feature that determines whether a young female larva becomes a worker or a queen is the early life diet, an example of developmental plasticity (Rembold

et al., 1974). Young female bees retain totipotency to become workers or queens up until approximately the third larval day after hatching, at which point their fate is more or less canalized based on the diet they received during this earliest period (Dedej et al., 1998). The rest of this dissertation will focus almost exclusively on worker bees, though, as they are the group that performs nearly all of the tasks that keep the colony functioning.

Worker bees display many complex behaviors within their natural behavioral repertoire. Workers will tend and feed the brood with special glandular secretions, care for the queen, build new wax honeycomb, process incoming pollen and nectar supplies for long-term storage, clean the hive, remove dead individuals, apply compounds with antimicrobial and "weatherproofing" properties to the nest, thermoregulate the colony, guard the nest entrance, and forage for multiple resources in a complex environment (Seeley, 1995). Each individual worker will temporarily specialize on an individual task at a given time. But she will progress through most of the tasks across her adult lifespan in an orderly pattern called "temporal polyethism" (Seeley, 1982). The youngest bees perform tasks closest to the center of the nest (such as brood care), middle-aged bees will perform tasks that are still within the nest but farther from the center (such as comb building and food processing), and the oldest bees will perform the riskiest tasks that take them outside of the colony (such as guarding and foraging) (Seeley, 1982). Most bees will do most of these tasks in approximately the order stated, although there is also flexibility in this progression to suit the needs of the colony, another example of behavioral plasticity (Robinson et al., 1992). Therefore, a worker honey bee needs to be equipped to handle a huge variety of cognitive demands across the span of her adult life. This stands in contrast to many other social insects with canalized task specializations and less behavioral

diversity within an individual (Schwander et al., 2005). In addition to this temporal polyethism, there are other small-scale social behaviors the bees will perform throughout their lives such as trophallaxis (food sharing) and allogrooming (Camazine et al., 1998; Kuswadi, 1992). This intricate division of labor is orchestrated through the use of sophisticated communication systems. Honey bees use many different pheromones as well as vibrational signals and even the famed honey bee "dance language" (Bortolotti & Costa, 2014; Schneider & Lewis, 2004; von Frisch, 1954). Features of the sensory systems the interpret these signals can be affected by health conditions as well, representing another route through which health and behavior affect one another (Farina et al., 2019; Favaro et al., 2022; Mondet et al., 2015). With all of this complexity, there is a wealth of opportunity to study even very sophisticated biological phenomena in these insects.

One potential complication in using a species as social as the honey bee to study behavior and health is that it can be difficult to disentangle individual versus group-level phenomena, particularly for socially relevant phenotypes. One prominent example can be found in aggression. Honey bees will aggressively defend the nest against both intruding insects and large predators (e.g. mammals) (Breed et al., 2004). They will perform characteristic attack behaviors (such as biting, swarming, chasing, bumping, and stinging) to drive away unwanted visitors (Breed et al., 2004). These aggressive behaviors are under the purview of a particular group of task specialists, namely guard and soldier bees (and to a lesser extent foragers, which are in the same age range as guards and soldiers) (Alaux et al., 2009; Breed et al., 1990). Aggressive nest defense is also a highly coordinated affair in honey bees, potentially involving up to thousands of individuals acting in concert thanks to alarm pheromones as well as visual and tactile cues (Hunt, 2007). As such, aggression

can be measured at the colony level or at the individual level with different assays, depending on the context one wishes to examine (Breed et al., 2004; Collins & Kubasek, 1982).

Aggression in honey bees is a highly complex phenomenon. It is affected by factors as diverse as genetics, recent disturbances, and the weather (Breed & Rogers, 1991; Rittschof & Robinson, 2013; Southwick & Moritz, 1987). Aggression also is strongly influenced by proximate social factors. For example, honey bees can occasionally be prompted to show aggressive behaviors when isolated, but they are much more likely to respond aggressively when they are within a social context (Moritz & Bürgin, 1987). The degree of aggression an individual bee will display is even subject to factors such as the aggressiveness of the bees she is immediately surrounded by, showing compensatory effects for group-level effort (Rittschof, 2017). Longer-time-frame effects are at play as well. Honey bee larvae who are raised in highly aggressive colonies become more aggressive as adults and show altered gene expression profiles compared to bees raised in more docile colonies, regardless of their genetic background (Rittschof et al., 2015; Rittschof et al., 2019). The aggression level of any individual bee is loosely associated with the aggression level of the colony she comes from, but the relationship is not perfect (Avalos et al., 2020). Thus, behavioral phenotypes such as aggression that manifest as both individual and collective behaviors provide interesting new avenues for research into behavioral plasticity, particularly given the well-documented association between aggression and health in these animals (Carr et al., 2020; Rittschof et al., 2015; Rittschof et al., 2019).

In addition to being an exceptional model system for studying behavioral plasticity and health, honey bees are also an important pollinator for human agriculture (Fikadu, 2019). Insect pollination improves yields of 75% of globally traded crop species, and much of this pollination is performed by managed honey bees (Klein et al., 2007). The billions of dollars in economic benefits they provide are under threat due to global honey bee declines that have intensified over the past several decades (Smith et al., 2013). These declines have been attributed to increased pesticide use, habitat loss and fragmentation, climate change, an aging population of beekeepers, and the combined effects of multiple health stressors such as pathogens and parasites (Kluser & Peduzzi, 2007). The importance of honey bees for human food security—and the mounting threats they face—make this work and others seeking to better understand health in these crucial pollinators all the more important and timely (Watanabe, 1994).

This project aims to further our understanding of how previous experiences can affect behavior and health in a highly social organism, the western honey bee. We examine this phenomenon across a range of behaviors from brood care to allogrooming. We additionally examine multiple timescales, from inputs in early life to transient social interactions in adulthood. Our specific aims are as follows:

1) Given the importance of early life nutrition, determine whether variation exists in the nutritional content of naturally occurring samples of the honey bee larval diet.
2) Assess whether key social interactions between nurse bees and the larvae they tend are affected by social signals and the colony-level social environment.

3) Examine whether worker bees show different antennal sensitivity to important social pheromones based on task specialization and the colony-level social environment.
4) Explore the relationship between social behavior and immune function by measuring changes in gene expression following an acute allogrooming event.

We begin this work with a literature review highlighting how insects in general can contribute to the study of developmental behavioral plasticity. We then move into separate chapters for the empirical work that addresses each of the above aims. Finally, we conclude by discussing overarching themes, implications for our findings, and suggestions for future work.

# CHAPTER 1. LITERATURE REVIEW: INSECTS PROVIDE UNIQUE SYSTEMS TO INVESTIGATE HOW EARLY LIFE EXPERIENCE ALTERS THE BRAIN AND BEHAVIOR

*Rebecca R. Westwick, Clare C. Rittschof*

This chapter was published in Frontiers in Behavioral Neurosciences in April, 2021.

Early-life experiences can have strong and long-lasting consequences for behavior in many animals. Determining which environmental inputs cause behavioral change, how this information becomes neurobiologically encoded, and the functional consequences of these changes remain fundamental puzzles relevant to diverse fields from evolutionary biology to the health sciences. Here we explore how insects provide unique opportunities for comparative study of developmental behavioral plasticity. Insects have sophisticated behavior and cognitive abilities, and they are frequently studied in their natural environments, which provides an ecological and adaptive perspective that is often more limited in lab-based vertebrate models. A range of cues, from relatively simple cues like temperature to complex social information, influence insect behavior. This variety provides experimentally tractable opportunities to study diverse neural plasticity mechanisms. Insects also have a wide range of neurodevelopmental trajectories while sharing many developmental plasticity mechanisms with vertebrates. In addition, some insects retain only subsets of their juvenile neuronal population in adulthood, narrowing the targets for detailed study of cellular plasticity mechanisms. Insects and vertebrates share many of the same knowledge gaps pertaining to developmental behavioral plasticity. Combined with

the extensive study of insect behavior under natural conditions and their experimental tractability, insects may be uniquely qualified to address some of the biggest unanswered questions in this field.

## 1.1 Introduction

Early-life experiences can have profound consequences for adult phenotypes, particularly behaviors (Beach & Jaynes, 1954), a phenomenon called developmental behavioral plasticity (sensu West-Eberhard, 2003, 2005). Although this phenomenon is well-established, its mechanistic basis remains a persistent research puzzle that touches many behavioral neuroscience disciplines and applications (Beldade et al., 2011; Reh et al., 2020; Snell-Rood, 2013). Brain development is fundamentally complex—it is a dynamic interaction between endogenous, gene-guided programs and environmental inputs (Boyce et al., 2020; Reh et al., 2020). Thus, determining how experiences are "embedded" requires knowledge at multiple levels of organization, from molecules to neural structure (Cardoso et al., 2015; Champagne, 2012; Curley & Champagne, 2016; Sinha et al., 2020). Moreover, individual differences can extend to peripheral tissues, which are also shaped by developmental experience and interact with the brain to influence adult behavioral expression (Fig. 1.1). Finally, in addition to triggering behavioral change, environmental conditions dictate the adaptive consequences of behavioral expression. Understanding these consequences may allow researchers to predict the types of experiences that cause lasting or transient behavioral impacts. However, adaptive consequences of behavioral expression are difficult to ascertain in traditional lab-based model systems alone (Yartsev, 2017).

Figure 1.1

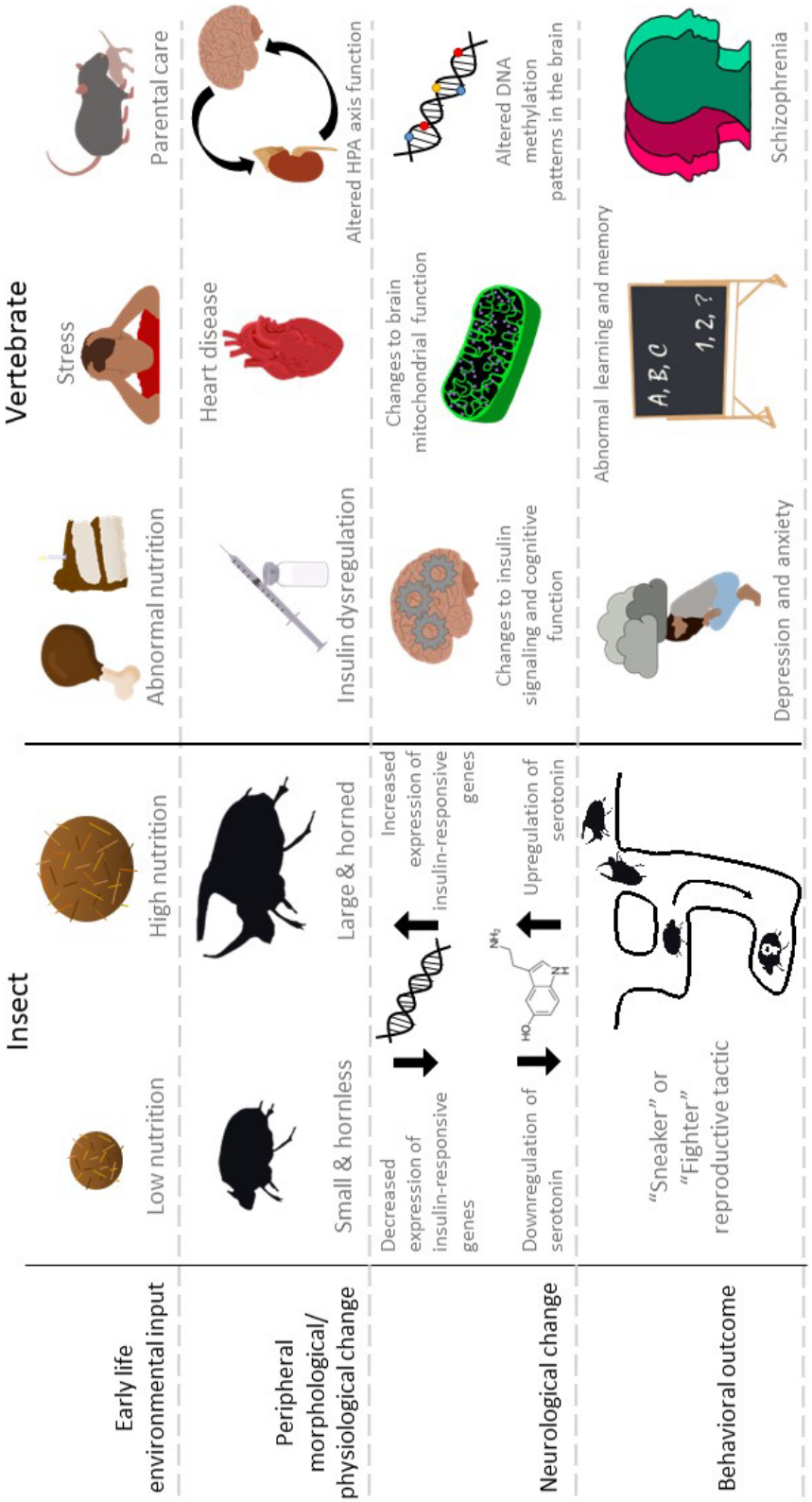

Insect
Vertebrate
Early life environmental input
Peripheral morphological/ physiological change
Neurological change
Behavioral outcome
Low nutrition
High nutrition
Abnormal nutrition
Stress
Parental care
Small & hornless
Large & horned
Insulin dysregulation
Heart disease
Altered HPA axis function
Decreased expression of insulin-responsive genes
Increased expression of insulin-responsive genes
Changes to insulin signaling and cognitive function
Changes to brain mitochondrial function
Altered DNA methylation patterns in the brain
Downregulation of serotonin
Upregulation of serotonin
"Sneaker" or "Fighter" reproductive tactic
Depression and anxiety
Abnormal learning and memory
A, B, C
1, 2, ?
Schizophrenia

**Figure 1.1: The impacts of early-life experiences extend beyond the brain to peripheral physiological systems and even body morphology in insect and vertebrate species.** The brain and peripheral systems interact to shape adult behavioral expression in ways that remain poorly understood. Though these brain-peripheral connections are common across animals including vertebrates, and specifically humans, some insects show particularly conspicuous and discrete changes in morphology, presenting interesting systems to investigate behavioral regulation. Moreover, despite the more noticeable phenotypic differences in some insects, there are examples of common regulatory mechanisms (e.g., insulin signaling) that underpin behavioral dynamics across the insect and vertebrate phylogenetic space. Left: In some beetles (Onthophagus spp.), males that are provided high amounts of nutrition during development emerge as large adults with horns (Emlen, 1997). Horns give males a benefit in competition over female mates, which nest in sub-terranean tunnels under dung piles (Moczek & Emlen, 2000). These morphological changes are associated with changes in brain insulin and serotonin signaling (Newsom et al., 2019; Snell-Rood & Moczek, 2012) and result in two distinct male reproductive tactics. Large, horned males will guard female tunnels and compete with other rivals, while small, hornless males dig side tunnels and sneak around large males to reach the female (Emlen, 1997; Moczek & Emlen, 2000). Right: In vertebrates, early-life nutrition, stress, and social interactions cause coordinated changes in peripheral physiological function (Avitsur et al., 2015; Barker, 1995; Champagne & Curley, 2005) as well as brain hormone signaling, bioenergetics, and gene regulation (Hochberg et al., 2011; Hoffmann & Spengler, 2018; Korosi et al., 2012). These changes can give rise to cognitive and mental health disorders (Avishai-Eliner et al., 2002; Chen & Baram, 2016; Sripetchwandee et al., 2018; Van Os et al., 2010).]

Fortunately, developmental behavioral plasticity occurs in animals as complex as humans and as simple as nematodes (Jobson et al., 2015; Kundakovic & Champagne, 2015). In this mini review, we explore how the insects are surprisingly well-suited to provide unique contributions to the study of this phenomenon. First, we highlight the strong ecological basis of insect behavior research (Schowalter, 2016), reviewing the exceptionally diverse systems available to explore the neurobiological basis of developmental behavioral plasticity in natural contexts with adaptive significance. Second, we provide an overview of the extensive examples of homology of function between insect and vertebrate nervous systems, despite their phylogenetic distance. We highlight the fact that a variety of mechanisms that embed developmental experience are broadly shared

across groups. We conclude that insects offer a fertile and exciting area of future comparative research that explores the complex relationships between early-life experiences and adult behavioral expression.

## 1.2 Insects as models for developmental behavioral plasticity in natural contexts

Extensive previous studies show that the developmental environment has diverse adaptive consequences for insect behavior. Such a perspective is valuable to behavioral neuroscience because environmental context defines the cues, sensory systems, and central processing dynamics that underpin behavioral change. Knowledge of environmental context may also be useful in establishing a general understanding of the types of conditions that give rise to transient versus lasting behavioral effects, a long-term goal in behavioral neuroscience. We highlight some of the established relationships between developmental experience and adult behavioral variation in insects, focusing on three major types of common environmental inputs: season, feeding experience, and interactions with other organisms.

### 1.2.1 Season

Many insects integrate seasonal cues during development and adaptively tune their adult behavioral expression to match environmental conditions (Benoit, 2010; Buckley et al., 2012; De Wilde, 1962). For example, in the butterfly *Bicyclus anynana,* males produce a costly nutritional gift they provide to females in order to improve their mating chances. The costs and benefits of this gift change from the wet to the dry season, and accordingly, males adjust their gift production and courtship efforts depending on developmental

moisture conditions (Prudic et al., 2011). In ground crickets (*Allonemobius fasciatus*), developmental temperature constrains male singing ability (Olvido & Mousseau, 1995), and as a result, females adjust their species-specific song preferences in response to their experience of temperature and day length during development (Grace & Shaw, 2004). Subtle differences in developmental temperature (e.g., developing in shaded versus sun-exposed shallow underground nests) can have profound behavioral impacts in female *Lasioglossum baleicum* bees; they shift from a cooperative reproductive tactic to a solitary one when developing in shadier locations (Hirata & Higashi, 2008). This selection of examples shows that the insects provide opportunities to investigate how simple developmental cues like temperature impact sophisticated phenotypes involving high level sensory integration and complex behaviors.

### 1.2.2 Feeding experience

Developmental feeding conditions can convey a variety of information. For example, because many insects are short-lived, developmental diet often predicts the state of nutritional resources available to the adult insect and even its offspring. Females of many insects, particularly moths, prefer to lay eggs on the same species they fed on during development (Petit et al., 2015), a phenomenon often referred to as Hopkins' Host Selection Principle (Hopkins, 1917). This pattern may minimize search time for suitable host plants for offspring. Though the mechanistic basis of this phenomenon remains controversial, experience-based developmental preferences for or against certain host plants or olfactory cues have been shown in multiple insect clades (Akhtar & Isman, 2003; Akhtar et al., 2009; ANDERSON & ANTON, 2014; Anderson et al., 2013; Barron, 2001;

Blackiston et al., 2008; König et al., 2015; Lhomme et al., 2017; Rietdorf & Steidle, 2002; Videla et al., 2010). Developmental feeding conditions can also indirectly signal the degree of intraspecific competition in the immediate environment, triggering mechanisms that alter myriad traits including adult body size, dispersal strategy, activity level, and exploratory behavior (Fig 1; Moczek & Emlen, 2000; Tremmel & Müller, 2012; Tripet et al., 2002).

Diverse neurobiological mechanisms are implicated in the response to developmental feeding experience. For example, plant volatile cues and the olfactory system play a strong role in butterfly and moth larval host plant identification (Petit et al., 2015). In other cases, including in some beetles, bees, aphids, and planthoppers, food intake itself is a cue leading to altered insulin and hormone signaling, which coordinate both peripheral and cognitive processes during development and throughout adulthood (Ament et al., 2008; Snell-Rood & Moczek, 2012; Zhang et al., 2019). More work is needed to understand how physiological processes like insulin signaling affect sensory perception and integration throughout adulthood, a topic that is currently of general interest in vertebrate cognitive neuroscience (Arvanitakis et al., 2020).

### 1.2.3 Interactions with other organisms

Other animals (but see also Schretter et al., 2018; Schwab et al., 2018 for the role of microbiota) commonly shape the insect developmental environment. For example, in a variety of insects, conspecific density and predation pressure induce developmental behavioral plasticity (Müller et al., 2016; Walzer & Schausberger, 2011). One famous case involves the transition from the solitary to gregarious phase in migratory locusts. Increased

frequency of physical contact during early life (a result of high conspecific density) gives rise to diverse morphological and behavioral changes, culminating in massive swarming events that disperse individuals to new locations with greater resources (Gillett, 1973; Simpson et al., 2001).

A variety of insect species (e.g., many ants, bees, wasps, termites) live in complex eusocial societies where certain members forego reproduction to help raise the offspring of their relatives (Oster & Wilson, 1978). Individuals of these species interact socially with conspecifics throughout life, including during development. Female caste differentiation, where females can develop into either a reproductive queen or a non-reproductive worker, is a well-studied example of developmental behavioral plasticity in these eusocial insects (Schwander et al., 2010). Queen/worker caste determination is typically a function of larval nutrition (at least in part) and mediated by adult "nurses" who provide food to larvae (Brian, 1956; Gadagkar et al., 1991; Liu et al., 2005; Page & Peng, 2001; Smith et al., 2008). In some eusocial insects, particularly ants, developmental dietary differences also give rise to behaviorally and morphologically distinct "soldiers" (female workers specialized for defense; Rajakumar et al., 2018).

There are other more subtle effects of the developmental social environment in eusocial insects (Miura, 2004; Traynor et al., 2014; Wang et al., 2014). For example, worker honey bees express different levels of defensiveness during adulthood depending on the defensiveness of the nestmates who rear them; this effect may be mediated by diet, but it is subtle enough that it does not alter body morphology (Rittschof et al., 2015). Adult wasps use vibratory signals directed at larvae, in combination with dietary interventions, to influence adult behavior, again without conspicuous changes in morphology (Jandt et

al., 2017). More primitive social insects also show effects of developmental social interactions. For example, in the twig-nesting small carpenter bee (*Ceratina calcarata*), a mother's removal from the nest during the larval stage eliminates maternal grooming activity and increases defensive and avoidant behaviors once offspring reach adulthood (Arsenault et al., 2018). Behavioral differentiation in developing insects involves a variety of cue types (e.g., nutrition, pheromone, vibratory, or tactile signals), often acting in combination, suggesting that diverse sensory and physiological systems are integrated to give rise to behavioral effects.

## 1.3 Homology in insect and vertebrate nervous system function and plasticity

Insects have a popular reputation of having simplistic, decentralized nervous systems (Schaefer & Ritzmann, 2001). While it is true that some processes are locally guided by "ganglia," semi-autonomous central nervous system components along the ventral nerve cord (Klowden, 2013), the brain is still required for sensory integration, decision-making, navigation, and learning (Pringle, 1940; Reingold & Camhi, 1977; Wessnitzer & Webb, 2006; Zill, 1986). Indeed, insects are capable of an impressive array of cognitive abilities, such as numeracy and social learning, because of their integrative brains (Alem et al., 2016; Avarguès-Weber, 2012; Chittka & Geiger, 1995; Coolen et al., 2005; Crist, 2004; Dyer, 1998; Giurfa et al., 1996; Giurfa et al., 2001; Pahl et al., 2013).

Insect brain structure and function is well studied (Ito et al., 2014), giving a strong basis to evaluate mechanisms of developmental plasticity from a comparative perspective. Extensive previous studies illuminate examples of homology of function with vertebrate systems (Simons & Tibbetts, 2019). Below we briefly review these general similarities,

and then focus on the specific neural mechanisms that encode developmental experience, many of which are also shared.

### 1.3.1 Homology of function between insect and vertebrate brains

Insect and vertebrate central nervous systems have similar functions (Kinoshita & Homberg, 2017) and many general features are shared, although notably, the evolutionary origin of these similarities remains controversial (Farris, 2008; Holland et al., 2013). For example, many of the same chemicals act as neurohormones and neurotransmitters, and even in conserved behavioral and cognitive contexts (Bicker et al., 1988; Byrne & Fieber, 2017; Osborne, 1996; Wu & Brown, 2006). In both vertebrates and insects including honey bees, bumble bees, fruit flies, and crickets, dopamine is involved in learning, novelty, reward prediction, and locomotion (Alem et al., 2016; Barron et al., 2008; Cohn et al., 2015; Felsenberg et al., 2018; Gadagkar et al., 2016; Hattori et al., 2017; Søvik et al., 2018; Terao & Mizunami, 2017). Likewise, serotonin modulates appetite, sleep, learning, social behavior, and aggression across a similar range of insect examples (Bubak et al., 2020; Rillich et al., 2018; Vleugels et al., 2015). Even insect-specific hormones have clear functional analogs in vertebrates. Insect juvenile hormone and vertebrate thyroid hormone both act through type II nuclear receptors, and they show similar growth and developmental functions (Charles et al., 2011; Flatt et al., 2006). Octopamine is an insect-specific neurohormone that is analogous to norepinephrine, and both compounds control stress response, motivation, and aggression (Alfonso et al., 2019; Prieto Peres & Valença, 2010; Roeder, 2005).

Beyond neurochemicals, recent studies suggest extensive homology between insect and vertebrate brain genome dynamics and protein function. Genes responsible for brain developmental patterning are surprisingly conserved (Lichtneckert & Reichert, 2005; Loesel, 2011; O'Connell, 2013; Reichert, 2009; Tessmar-Raible et al., 2007), and there is even evidence for functional conservation of genes associated with complex behaviors like territorial aggression, foraging, and brood care (Rittschof et al., 2014; Saul et al., 2019; Shpigler et al., 2018; Toth & Robinson, 2007; Toth et al., 2014). Cell types in the brain show similarities in structure and function. Like vertebrates, insect brains contain neurons and various types of glia (Losada-Perez, 2018), and the metabolic relationships between these cell types are similar across groups (Rittschof & Schirmeier, 2017). Neural activity is well-known for its energetic demands (Niven & Laughlin, 2008; Peters et al., 2004), and insects and vertebrates share some neural adaptations to high energy need (Robertson et al., 2020) and increased cognitive demands; the latter even shows a similar developmental basis (Farris, 2008).

Despite extensive similarities, insects do show some profound differences in nervous system structure and function compared to vertebrates. For example, insect neurons are unmyelinated, they have different classes of olfactory and photoreceptors compared to vertebrates, and neuronal polarity is often different (Albert & Kozlov, 2016; Chittka & Niven, 2009; Gutierrez et al., 2011; Kaupp, 2010; Rolls & Jegla, 2015). Another conspicuous difference between insects and most vertebrates is the structure of early-life development (Fig. 1.2), including the somewhat extreme behavioral and morphological changes that occur during insect metamorphosis. Metamorphosing amphibians and fish are a notable exception within vertebrates and provide an exciting avenue for comparative

work (Gilbert et al., 1996; Heyland & Moroz, 2006; Lowe et al., 2021; Shi, 2013). As with outward appearance, the structure and function of the nervous system can change dramatically during metamorphic developmental transitions in insects (Gilbert et al., 1996; Weeks & Truman, 1986; Wolbert & Kubbies, 1983). For instance, butterflies transition from relatively sessile plant-eating caterpillars to flighted adults with distinct diets, behavioral traits, sensory structures, and motor and cognitive capabilities (André, 1991; Ebenman, 1992). About 80% of all insect species (including ants, bees, wasps, butterflies, beetles, and flies among others) experience this extreme form of metamorphosis ("complete metamorphosis", Rolff et al., 2019). Most other insects experience incomplete metamorphosis, where the pupal stage is absent and the body plan in early life is more similar to that of the adult form (except for the absence of wings). Notably, some of these species still show radical differences in life history between juvenile and adult stages (Corbet, 1957; Gabbutt, 1959). The variation in development patterns in insects make them exciting but perhaps challenging subjects for comparative study of developmental behavioral plasticity.

Figure 1.2

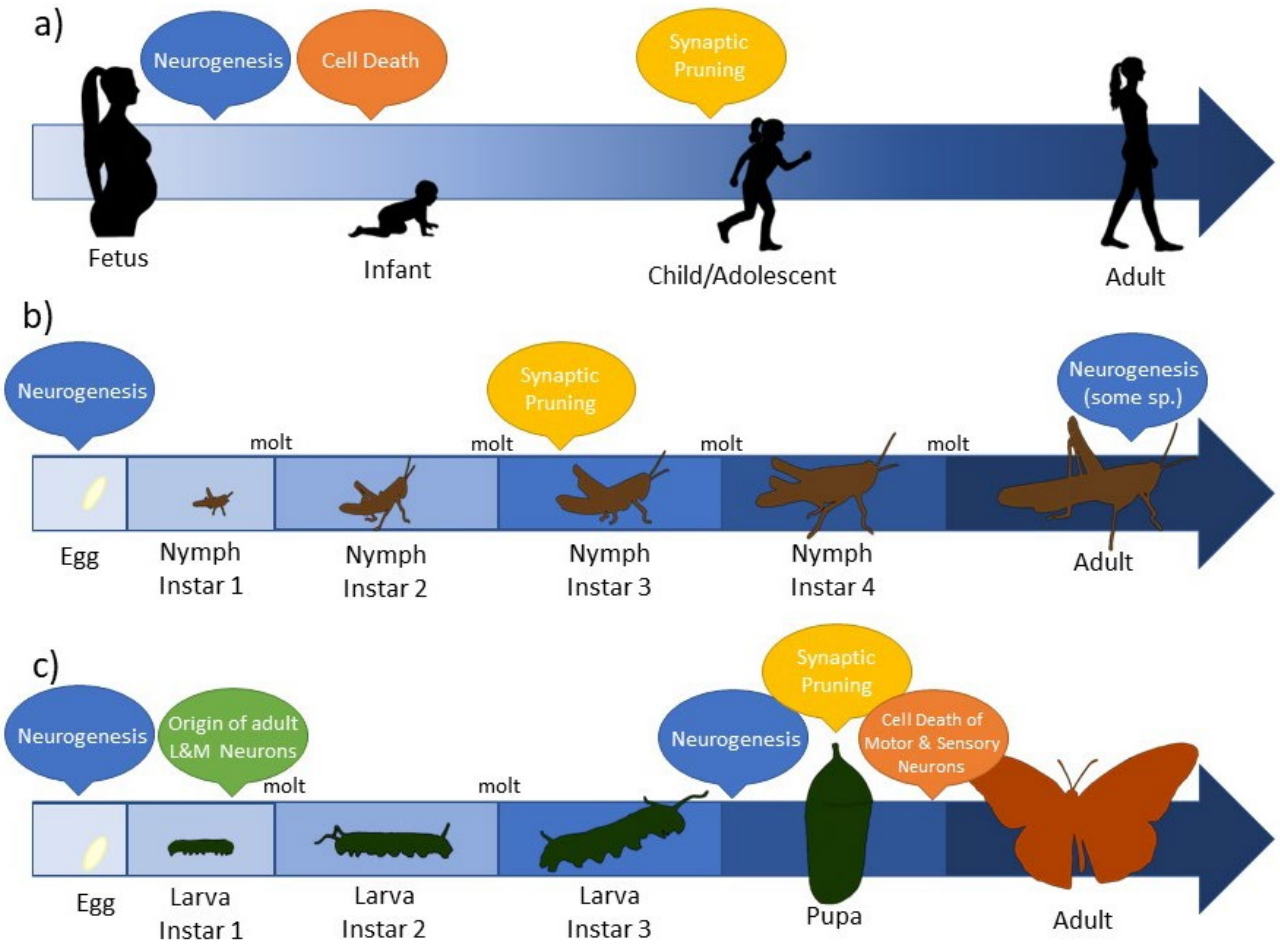

a)
Neurogenesis
Cell Death
Synaptic Pruning
Fetus
Infant
Child/Adolescent
Adult
b)
Neurogenesis
Synaptic Pruning
Neurogenesis (some sp.)
molt
molt
molt
molt
Egg
Nymph Instar 1
Nymph Instar 2
Nymph Instar 3
Nymph Instar 4
Adult
c)
Neurogenesis
Origin of adult L&M Neurons
Synaptic Pruning
Neurogenesis
Cell Death of Motor & Sensory Neurons
molt
molt
Egg
Larva Instar 1
Larva Instar 2
Larva Instar 3
Pupa
Adult

**Figure 1.2 Patterns of development, specifically the timing of neurobiological events, vary across vertebrates and insects.** Although insects and vertebrates show remarkable overlap in the types of mechanisms that characterize brain development and entrain early-life experience (Bello et al., 2008; Luo & O'Leary, 2005; Pearson, 1993; Reh & Cagan, 1994; Salzberg & Bellen, 1996; Watson, 1992), the progression of early-life, and specifically the timing of events like neurogenesis, programmed cell death ("Cell Death"), and synaptic pruning, differs markedly across these groups. **A)** Most vertebrates show gradual changes in body size and tissue morphology. In the brain, they experience massive neurogenesis early in life followed by cell death and pruning through adolescence and early adulthood (Watson et al., 2006). Notably, more limited neurogenesis also occurs during adulthood (Zhao et al., 2008). **B)** Some insects also show a pattern of gradual development (called "incomplete metamorphosis"), where juvenile stages resemble the basic body plan of adults. However, these insects still shed their exoskeletons in order to grow, and as a result, they transition through distinct developmental stages. Relatively little is known about neurobiological events in these species, although there is evidence of extensive neurogenesis both prior to egg hatch and during adulthood (Cayre et al., 1994). There is also evidence for synaptic pruning dynamics that resemble vertebrate mechanisms (Lnenicka & Murphey, 1989). **C)** The majority of insects (~80% of species) show a pattern of complete metamorphosis, where life stages have distinct morphologies, and adult behaviors and body plans vastly differ from juveniles. Data from several representatives of this group again suggest multiple periods of neurogenesis, both early in life and during the pupal stage (Booker & Truman, 1987; Truman & Bate, 1988). Interestingly, the timing of neurogenesis and programmed cell death and the retention of neurons through the life stages is brain region (and thus, functionally) specific (Tissot & Stocker, 2000; Wegerhoff, 1999). For example, a small number of neurons responsible for learning and memory originate early in the larval period and persist through adulthood, but most motor and sensory neurons are completely remodeled during the pupal phase (Cantera et al., 1994).

Despite their developmental complexities, one unique benefit to insect study is that in some species, particularly those that undergo complete metamorphosis, only a subset of neurons is retained between the juvenile and adult stages (Fig 2, Cantera et al., 1994; Tissot & Stocker, 2000; Wegerhoff, 1999). This feature narrows the target populations for studies of early-life environmental effects. For example, in the sensory integration and learning and memory centers of the brain (primarily the "mushroom bodies"), adult neurons typically originate during early larval life, suggesting adequate opportunity to retain environmental information into adulthood; this is in contrast to sensory neurons, which are completely distinct between the larval and adult stages (Cayre et al., 1994; Tissot &

Stocker, 2000). Moreover, even though the degree of neuronal remodeling may be relatively extreme in insects compared to vertebrates, the components of the remodeling process closely resemble the types of developmental changes that also occur in vertebrates (Bello et al., 2008; Luo & O'Leary, 2005). For example, analogous to developing vertebrates, different neuron populations in circuits associated with learning and memory display a coordinated process of pruning and regrowth during metamorphosis in *Drosophila melanogaster* (Mayseless et al., 2018; Spear, 2013). These features of insect neurodevelopment provide unique opportunities to study the complex neural mechanisms of developmental behavioral plasticity in careful detail.

### 1.3.2 Homology of function in neural mechanisms that encode developmental experience

Early-life cues change adult behavior by persistently altering the structure and/or function of the nervous system (Odeon et al., 2013). Though the precise dynamics of these changes remain poorly understood in any system, in general terms, known mechanisms are similar comparing vertebrates to insects (Pearson, 1993; Reh & Cagan, 1994; Salzberg & Bellen, 1996; Watson, 1992). Major categories of mechanisms include epigenetic modifications, changes in the quantity of neurochemicals and/or their receptors, and brain structural changes (Elekonich & Robinson, 2000; Fahrbach, 2006; Glastad et al., 2019; Kretzschmar & Pflugfelder, 2002; Schoofs et al., 2017). These mechanisms are not mutually exclusive, and one long-term challenge in behavioral neuroscience for insects and vertebrates alike is to understand how these mechanisms are integrated to alter dynamic behaviors (Wolf & Linden, 2011). However, here we highlight some known insect

examples of epigenetic, neurochemical, and structural mechanisms that encode developmental experience.

Chemical modifications to brain DNA are proposed to be critical mediators of early-life effects on adult behavior in vertebrates (Aristizabal et al., 2019). DNA methylation and histone post-translational modifications are the most well-studied among these mechanisms (Paredes et al., 2016; Smallwood & Kelsey, 2012). Not all insects possess appreciable levels of DNA methylation (Deobagkar, 2018; Deshmukh et al., 2018), but some, including many social insects, do (Li-Byarlay, 2016; Yagound et al., 2020). Some studies show that developmental experience-induced changes in DNA methylation impact adult behavioral phenotypes (Alvarado et al., 2015; Linksvayer et al., 2012; Patalano et al., 2012; Weiner & Toth, 2012; Yan et al., 2014). For example, the variation in larval diet that gives rise to queen versus worker female honey bees acts at least in part through DNA methylation changes in both the head and peripheral tissues (Kucharski et al., 2008; Shi et al., 2011; Wang et al., 2020). Similarly, studies in termites and locusts demonstrate a relationship between differential DNA methylation and developmentally induced adult behavioral variation (e.g., in the solitary versus gregarious phases of migratory locusts, Lo et al., 2018). Other known epigenetic mechanisms also play a role in developmental behavioral plasticity in insects, including histone modifications and long non-coding RNAs (Glastad et al., 2019; Simola et al., 2016).

The relationship between brain epigenetic modifications and gene expression patterns varies across species and is not well-understood. For example, whereas DNA methylation in gene regulatory regions tends to suppress gene expression in vertebrates, in insects, gene body methylation, which is thought to regulate alternative splicing, is more

common (Feng et al., 2010; Glastad et al., 2014; Schmitz et al., 2019; Zemach et al., 2010). Furthermore, some studies have shown surprisingly weak relationships between DNA methylation dynamics and behavioral expression (Herb et al., 2012; Libbrecht et al., 2016). More data is necessary to understand how DNA methylation dynamics correspond to both gene expression dynamics and behavior (Flores et al., 2012; Jeong et al., 2018; Li-Byarlay, 2016), including whether the presence and degree of DNA methylation and other epigenetic modifications predict capacity for behavioral plasticity (Kapheim et al., 2015; Lo et al., 2018). These are general challenges facing vertebrate research as well (Di Sante et al., 2018), which could benefit from a comparative approach.

The developmental environment can cause lasting behavioral effects by altering neurochemical processes, e.g., circulating levels of hormones and neurotransmitters in the central nervous system. For example, changes in brain insulin, juvenile hormone, prothoracicotropic hormone, octopamine, and serotonin signaling are prominent correlates of insect developmental behavioral plasticity (De Wilde & Beetsma, 1982; Erion & Sehgal, 2013; Moczek & Emlen, 2000; Newsom et al., 2019; Paulino Simões et al., 1997; Rachinsky, 1994; Snell-Rood & Moczek, 2012). These chemicals impact behaviors like aggression, gregariousness, feeding, locomotion, and nonaggressive social interactions (Anstey et al., 2009; Erion & Sehgal, 2013; Iba et al., 1995) in a number of species, including the cricket and locust examples above. The degree to which neurochemical systems comparably regulate behaviors across vertebrates and invertebrates is a matter of debate (Bubak et al., 2020), and thus an important area of on-going study, especially in the context of developmental behavioral plasticity.

A final common way the developmental environment affects the nervous system is through brain structural changes (Hall & Tropepe, 2020; Saleh et al., 2017; Teicher et al., 2016). For example, in flies, high conspecific density during development results in larger mushroom bodies and enhanced olfactory processing abilities (Heisenberg et al., 1995). Similar conditions in wasps lead to increased overall adult brain size, and larger-volume mushroom bodies and regions required for visual processing (Groothuis & Smid, 2017). Gregarious locusts have larger integrative mushroom bodies, while solitary individuals show neural adaptations associated with enhanced sensory sensitivity (Ott & Rogers, 2010). Female social insects often show variation in relative brain region size as a function of behavioral specialization (Lucht-Bertram, 1961; Muscedere & Traniello, 2012; Page & Peng, 2001; Vitt & Hartfelder, 1998; Wheeler & Nijhout, 1984). Insect and vertebrate nervous systems not only exhibit many of the same developmental plasticity mechanisms, but they also face many of the same conceptual challenges associated with connecting developmental experience to behavioral expression. These extensive similarities suggest many potential benefits to comparative study.

## 1.4 Discussion

Predicting, and in some cases changing, adult behavioral effects of early-life experience are challenges relevant to diverse fields of behavioral neuroscience (Beldade et al., 2011; Bryck & Fisher, 2012; Danese, 2020; Reh et al., 2020; Snell-Rood, 2013; Stamps & Biro, 2016; West-Eberhard, 2003). Behavioral effects of early-life experience are commonplace among animal species, presenting the opportunity to use comparative approaches to identify the general principles of developmental behavioral plasticity. Many fundamental questions that are common to both insects and vertebrates remain to be

resolved, for example, how the brain integrates early-life experience across multiple levels of organization, and whether specific mechanisms like DNA methylation universally predict long term behavioral impacts. Moreover, it remains unclear how developmental experiences are integrated with other sources of information (e.g., genetic variation, parental transgenerational effects) that also influence behavior (Dall et al., 2015; Rösvik et al., 2020; Stamps & Frankenhuis, 2016; Stein et al., 2018), and whether these outcomes can be modified by additional information later in life. Though these sources of complexity apply to both insect and vertebrate species, certain characteristics of insects, like their relatively short lifespans, may alter the ecological selection pressures that shape information integration. With respect to the evolution and expression of behavioral plasticity, diverse comparative approaches may illuminate both broad, general features and taxon-specific patterns.

In insects, studies of behavioral plasticity largely focus on processes during the adult stage, and although many patterns of nervous system development are known (Awasaki et al., 2008; Cayre et al., 2000; Hähnlein & Bicker, 1997; Prillinger, 1981; Rospars, 1988), precisely how these patterns respond to early-life environmental stimuli remains poorly understood. However, the deep research history of insects in natural ecological contexts provides diverse, tractable systems for future work that fills this research gap. The developmental environment, including simple abiotic factors like temperature and moisture, impacts a variety of sophisticated behaviors from dispersal patterns (Alyokhin & Ferro, 1999; Benard & McCauley, 2008; Zera & Denno, 1997) to social and reproductive tactics (Emlen, 1997; Kasumovic & Brooks, 2011; Łukasik, 2010; Radwan, 1995; Taborsky & Brockmann, 2010). Thus, in controlled but environmentally

relevant experiments, it is possible to assess how specific types of developmental inputs shape both sensory and integrative processes (Anton & Rossler, 2020; Fernandez et al., 2020; Gonzalez-Tokman et al., 2020) relevant to many different behavioral phenotypes. In addition, the short generation time of insects is ideal for life-long studies of behavior.

On the neurobiological level, developmental behavioral plasticity in insects is mediated through familiar neural plasticity mechanisms like epigenetic modifications, neurochemical changes, and changes to neural structure (LeBoeuf et al., 2013). Some of these mechanisms can, and have been, explored in the context of traditional learning and memory frameworks, which also are well established in insects (Alloway, 2015; Blackiston et al., 2008; Tan et al., 2015; Tully et al., 1994; Yang et al., 2012). Though most learning and memory research has focused on dynamics during the adult stage (Fahrbach et al., 1998; Jernigan et al., 2020; Li et al., 2017; Ravary et al., 2007), many insights from this work are likely applicable to the pre-adult life stages as well. Moreover, in what may be the majority of insect species, only a subset of the brain survives the transition from the juvenile life-stage to adulthood, presenting a narrow range of target areas in which to carefully investigate how neural plasticity mechanisms give rise to complex behaviors. However, some challenges to comparative work remain. For instance, it is unclear which insect life stages are comparable to the early-life timeframe in vertebrates, or whether retention of early-life effects in insects is fundamentally constrained by their extensive morphological and neurobiological remodeling (Vea & Minakuchi, 2020).

Despite these challenges, insects have a history of contributing surprisingly general insights into complex behavioral phenotypes relevant to vertebrate species. For example, eusocial insects present detailed systems to address general neurobiological principles of

developmental behavioral plasticity in the context of complex social living. Because insect societies show patterns of organization that can be generalized to other social species (Bonner, 2004; Ireland & Garnier, 2018; Seeley, 1995), they have tremendous promise for investigating both the causes and consequences of developmental plasticity in vertebrates. This comparison may even extend to humans, where many persistent effects of the early-life environment on behavior and mental health are social in nature (Miller et al., 2009; Nothling et al., 2019). It is possible that behavioral plasticity in social contexts has unique neurobiological features (Taborsky & Oliveira, 2012), and social insects will continue to serve as excellent models to examine this idea.

Although this review is specifically focused on insect contributions to behavioral neuroscience in a comparative framework, the uniqueness of this animal group, and its ecological and economic importance, cannot be overstated. These aspects provide further motivation for study of developmental behavioral plasticity in this group. Many bee species are important agricultural pollinators (Reilly et al., 2020; Winfree et al., 2011). The ongoing locust outbreak in East Africa is anticipated to cause enormous economic loss and endanger food security (Peng et al., 2020). Many agricultural pests are metamorphosing insects with destructive larval feeding stages (e.g., beetles and moths). Understanding the natural history of these organisms, as well as the range of neural and behavioral responses to developmental experience (De França et al., 2017; Desneux et al., 2007; Haynes, 1988; Müller, 2018; Sehonova et al., 2018) will improve environmental management in addition to deepening our understanding of the general principles of developmental behavioral plasticity.

# CHAPTER 2. A NUTRITIONAL SECRETION, WORKER JELLY, SHOWS HIGH AMONG-COLONY VARIATION IN MACRONUTRIENT CONTENT IN HONEY BEE (*APIS MELLIFERA*) COLONIES.

*Rebecca R. Westwick, Clare C. Rittschof*

Nutrition is critically important for an organism's survival and health throughout its lifetime. This fact is particularly true during the early life period of growth and development. Many organisms help buffer against the devastating effects of early life malnutrition by provisioning offspring. One of the more extreme forms of provisioning involves an animal creating nutritional secretions from specialized body structures to nourish their young. These processes have been well-characterized in vertebrates such as mammals and birds, but some invertebrates provision their young with secretions as well. The honey bee (*Apis mellifera*) is one such invertebrate. Here, we investigate the within- and among-colony variation in the macronutrient content of "worker jelly," a secretion that is synthesized by adult worker bees specifically to feed the young, collected from cells with age-matched larvae. Despite the known negative effects of malnutrition in young honey bee larvae, we find that there is high inter-colony variation in the total food and in the macronutrient contents of samples from different colonies. Furthermore, this variation is not well explained by colony aggression level, colony site, or colony genetic strain.

## 2.1 Introduction

The early life period is crucial for setting multiple aspects of the health and development of an organism (Fagundes et al., 2013). Early life nutrition in particular has long-lasting consequences for an organism's growth and survival, as deficits during this

time often cannot be fully compensated for later (Alippi et al., 2002; Bedi et al., 1982; Guthrie & Brown, 1968). One way that organisms have evolved to hedge against this risk is via parental care, with adults helping to provision the young and ensure adequate nutritional intake during this vulnerable stage (Beekman et al., 2019). Provisioning can involve adults simply assisting their offspring in finding naturally available nutritional resources, such as convict cichlids that will dig and lift leaves to help their offspring access food (Wisenden et al., 1995). Another style of provisioning involves the adult carers gathering and then processing the nutritional resources before feeding the young, such as birds that will regurgitate partially digested stomach contents for their young (Duffey, 1951). The most extreme form of provisioning, however, involves adult animals developing specialized body structures to synthesize and secrete nutritional products tailored for their young, exemplified by milk produced by mammals (Oftedal, 2012). Nutritional secretions are seen in a wide variety of taxa, though, such as pigeons, bony fish (ectodermal feeding), and some social insects among others (Blumer, 1982; Mas & Kölliker, 2008; Patel, 1936; Snir et al., 2022; Stay & Coop, 1974). These organisms face the challenge of synthesizing a product that will meet all (or nearly all) of the changing nutritional needs of a developing young organism through different growth stages (Andreas et al., 2015). Thus, the contents of nutritional secretions often change over time to meet the stage-specific needs of the offspring (Gil & Sanchez-Medina, 1981; Jenness, 1979).

In addition to the variation in nutrients seen over time in nutritional secretions, strong inter-individual variation is also relatively common where it has been examined (Amigo & Fontecha, 2011; Kamelska et al., 2012; Khan et al., 2013; Laben, 1963; Michaelsen et al., 1990; Qin et al., 2019). This finding is somewhat surprising, as most

young organisms within a given stage would be expected to have relatively similar nutritional needs and therefore a similar optimal nutritional intake profile (Jenness, 1979). Indeed, even variation in individual human milk oligosaccharides has been correlated with a range of negative health outcomes in human infants (Doherty et al., 2018). But these studies have largely been conducted in mammalian systems, where one parent is responsible for all of the nutritional resources that each offspring receives. Some social insects (such honey bees), on the other hand, distribute care of the young among many hundreds or even thousands of adults who are oftentimes the siblings of the young being reared rather than a parent. It is possible that this distribution of care results in a buffering against individual variation in nutritional secretions (or potentially heightens variation), but this idea remains unexplored.

Honey bees provide an excellent system to examine natural variation in the nutritional profile of nutritional secretions. The young larvae are tended by a group of adult workers called "nurses" that are temporarily specialized on brood care, and hundreds to thousands of workers will actively perform nursing within a colony at a given time (Johnson, 2008; Seeley, 1982). These nurse bees are the older half and full siblings of the larvae they tend (Kennedy, 2021). Adult worker bees have structures called hypopharyngeal glands located in their head capsules where the "worker jelly" that is fed to the worker larvae is synthesized, and these glands are highly enlarged in active nurse bees (Knecht & Kaatz, 1990). Though previous studies have determined the basic components of worker jelly, these studies have often not included a range of samples that would sufficiently allow for detection of natural variation within and among honey bee colonies (see "*General Observations*"). In addition, worker jelly is far less examined than

its counterpart, royal jelly, the secretion that is fed exclusively to larvae destined to become future queen bees (Ramanathan et al., 2018). It is known that nurse bees will alter the nutritional profile of the worker jelly based on the age of the larva being fed (Brouwers et al., 1987). But whether stable variation exists within or among different honey bee colonies *within* a given larval stage has been poorly studied, much less the consequences of any natural variation that might exist. Understanding the typical larval honey bee diet is a critical missing component in the link between the environmental resource conditions that determine what nutritional element flow into the hive and colony health.

In the current study, we assessed the degree of natural variation in honey bee worker jelly within and among colonies. We hypothesized that overall variation would be relatively low, given that worker jelly is a processed product created by nurses that can act as a buffer to fluctuations in environmental resource conditions. For example, under natural conditions, if there is a scarcity of nutrients, nurses will first break down their own body stores to continue producing worker jelly (Haydak, 1935). But if the food shortage continues, workers will typically cannibalize larvae rather than produce sub-par brood (Imdorf et al., 1998; Schmickl & Crailsheim, 2001). Studies also suggest that the type of pollen consumed can affect the initial development of the hypopharyngeal glands in nurses, but that it does not affect the amount of proteins in royal jelly (Lan et al., 2021; Omar et al., 2017; Pattamayutanon et al., 2018; Renzi et al., 2016). And finally, foragers bring in the pollen that supplies many of the critical nutrients that nurses consume to create worker jelly—and these foragers have preferences for particular types of pollen. Foragers sometimes prefer pollens that are rich in particular nutrients such as proteins, but they can also calibrate their foraging effort to the nutritional value of the pollen they collect

(collecting a greater quantity of pollen from nutrient-poor sources) and can shift their intake to make up for nutritional deficits of particular amino acids and essential fatty acids (Campana & Moeller, 1977; Ghosh et al., 2020; Hendriksma & Shafir, 2016; Liolios et al., 2015; Zarchin et al., 2017). These findings could indicate that there are evolutionary guardrails around some qualities of worker jelly: foragers target the nutritional intake for the colony and the nurses physiologically process the nutrients before they become worker jelly, which supports our hypothesis that nutritional components for worker jelly should be relatively consistent (particularly for proteins).

Given that nutritional secretions in other animals show individual variation (as described above), though, we also aimed to examine different factors that could be driving any natural variation in worker jelly nutritional content, should it exist. A recent study by members of our group found that the social context a honey bee larva is raised in affects her behavior and health in adulthood. Larvae crossed-fostered in high-aggression colonies were more aggressive, healthier, and had distinct transcriptomic profiles as adults compared to larvae cross-fostered in low-aggression colonies (Rittschof et al., 2015; Rittschof et al., 2019). The mechanisms that drove these changes were not examined in these studies. Nutrition being such a vital piece of a larva's development, we first examined whether these adult outcomes that are correlated with larval social environment could be driven by differences in larval diet between high- and low-aggression colonies. This hypothesis is further supported by the known relationship between aggression and foraging ability in honey bees (Wray et al., 2011). We additionally examined two alternative hypotheses: that variation in larval diet is associated with colony site (as the location will determine what food resources the colony has available for foraging) as well as the colony

genetic strain (in case differences in worker jelly nutritional content are due to factors intrinsic to the nurses that synthesize it). We chose to focus our analyses on total food and three critical macronutrients (proteins, lipids, and carbohydrates). Negative effects of larval malnutrition have typically been demonstrated in artificial experiments that involve broad changes in nutritional availability, either at the level of whole food sources (such as whole food or whole pollen restriction) or at the level of macronutrients (such as changing the amount of proteins available in an artificial diet), demonstrating the importance of these larger components (Aupinel et al., 2005; Brodschneider et al., 2009; Daly et al., 1995; Eishchen et al., 1982; Jay, 1964; Mattila & Otis, 2006; Scofield & Mattila, 2015).

## 2.2 Methods

### 2.2.1 Sample collection

We collected worker jelly from honey bee colonies in central Kentucky, USA. These colonies had mostly been installed as packages at the beginning of the season with strains advertised as “Italian” and “Russian Hybrid”(Schoolhouse Bees, Covington, KY). Remaining colonies were of mixed local genetic stock. Colonies were situated at three sites near Lexington, KY, USA. Two of the sites were approximately 1 mile apart (“Alpha” and “Beta”) and the third was approximately 9 miles away from the other two (“Gamma”), allowing us to examine the effects of colony site on nutritional components. Between June 29-July 21 of 2019, we performed colony-level aggression assays using previously established methods (Alaux & Robinson, 2007; Rittschof et al. 2015). Briefly, photographs of the baseline activity level were taken at the entrance of every colony. We pipetted 3uL of a 1:10 dilution of isopentyl acetate (an important component of the honey bee alarm

pheromone) in mineral oil onto a strip of filter paper. We placed the strip of paper at the entrance of the colony. We then took a second photograph 1 minute later. The raw aggression score was calculated as the difference between the number of bees at the entrance after application of isopentyl acetate and the baseline activity level. The highest-aggression and lowest-aggression colonies were chosen for worker jelly collections, balanced across sites (N=18 total colonies, 9 per aggression level, with 3 high-aggression and 3 low-aggression colonies at each site). Though genetics play a role in many honey bee traits, these patterns are not always clear or consistent at the level of genetic strain, and indeed, colonies did not clearly separate into high and low aggression group based on strain (see RESULTS). Nonetheless, we evaluate a role for strain in dietary variation (see RESULTS, "*Alternative hypotheses for explaining variation in worker jelly nutritional content between colonies")*.

The contents of the worker jelly are known to change with the age of the larvae (Brouwers et al., 1987). We therefore sampled worker jelly from age-matched larvae. For each selected colony, we caged the queen with an empty honeycomb frame for 24 hours to allow her to lay eggs. The cage fit one standard frame (~19 in. x 1-1/16 in. x 9-1/8 in.) and was covered by a grate that was large enough to allow workers to pass in and out freely while being small enough to contain the queen. After 24h, the queen was removed from the cage and returned to the colony, and the frame was re-caged and placed back in the same colony to prevent further laying by the queen. This approach generates dozens to hundreds of eggs that range in age from 0 to 24 h old. The nurse bees begin making inspection visits even during the egg stage. Nurses continue making inspections and begin making feeding visits to deposit worker jelly almost immediately once the larva hatches.

For our experiment, we removed the frame from the colony two days after the eggs hatched (96-120 hours post-laying depending on exactly when in the 24 h period the egg was laid) between 9:00 and 11:00 and brushed off all bees. We took the frame into a cool, shaded location away from the colony and lightly covered the frame with damp paper towels until extraction began to prevent larva and worker jelly desiccation. At the beginning of extraction, we would remove the paper towels and haphazardly selected approximately 27 cells from one side of each frame, covering cells from the entire laying area, excluding any cells where the larvae were markedly larger or smaller or had any abnormalities (N=467 total samples collected; some colonies had fewer than 27 samples collected due to an insufficient number of useable samples). This process should encompass a similar range of larval ages per colony since the queen lays eggs in a predictable pattern, such that nearby eggs are more similar in age. All samples were collected over 8 collection days that fell within the span of 23 calendar days to minimize potential seasonal effects. For example, crop bloom cycles are usually several weeks at a minimum and climatic patterns would be relatively constant over a timeframe this short, and honey bees in our area are typically active for 6-7 months (NASS, 2023; NWS, 2023). Each collection day involved taking samples from two or three colonies, always from the same yard, and included at least one high- and one low-aggression colony at a time. See Appendix 1 for a table displaying colony collection order (Table A1S1).

Honey bee larvae sit in the base of honeycomb cells in a small pool of worker jelly. To extract the worker jelly, we pipetted 100uL deionized water into the cell to float the larva to the top. We carefully removed the larva with a grafting tool and saved it in a separate tube, kept at -80ºC. We then pipetted an additional 100uL of deionized water into

the cell. We drew the slurry of water and worker jelly in and out of the pipette several times to mix it and to loosen the food from the wax of the cell. We pipetted the mixture into a 1.5mL microcentrifuge tube (Thomas Scientific), where it was stored at -80ºC until chemical processing.

### 2.2.2 Sample processing

Worker jelly has a gelatinous texture and is comprised of water, proteins, lipids, carbohydrates, and other material such as micronutrients and nondigestible material. In order to homogenize the worker jelly samples and break up clumps, we sonicated the samples for 5 minutes on 100% power on a Misonix S-4000 (Newton, CT) cup horn sonicator. We then determined the total wet and dry mass of each worker jelly sample from a 20uL aliquot.

### 2.2.3 Protein assay

We calculated the amount of proteins per sample from a unique aliquot (separate from the aliquot used for lipid and carbohydrate determinations, see below) using a bicinchoninic acid (BCA) assay (Pierce™ BCA Protein Assay Kit, 23227). The assay was carried out according to the manufacturer's instructions for a microplate preparation. Bovine Serum Albumin (BSA) 2ug/uL was used as the standard. We ran the final product of the assay in triplicate for each sample and used the median value for analysis. We ran the samples in a random order to mitigate any potential plate effects.

### 2.2.4 Lipid and carbohydrate assays

Lipid and carbohydrate levels were measured from a separate aliquot on which we performed combined chloroform-methanol extraction and fractioning (described in Foray et al. 2012). We performed a sulfo-phospho-vanillin assay on the chloroform fraction to determine the amount of lipids per sample. Commercial vegetable oil suspended in chloroform (1ug/uL) was used as the standard. An anthrone-sulfuric acid assay was used on the methanol fraction to determine carbohydrate concentrations. Anhydrous glucose dissolved in deionized water (1ug/uL) was used as the standard for this assay. Both assays were carried out in accordance with the protocol outlined in (Foray et al., 2012). As in the protein assays, all samples were run in a randomized order and were measured in triplicate once the colorimetric change had occurred. The median value of each triplicate was used for analysis.

### 2.2.5 Statistical analyses

We performed statistical analyses with R version 4.1.2 (R Core Team, 2021). Our analyses primarily focused on total dry weight, total proteins/lipids/carbohydrates, and mass-corrected (fractional) proteins/lipids/carbohydrates as response variables. For explanatory variables, we examined colony aggression, site, and colony genetic strain. We assessed aggression three different ways: as a binary variable (high/low), as a ranked variable (1-18), and as a continuous variable using raw aggression scores (described above). We used the “aov” function from the “stats” package to run ANOVAs to calculate

among-colony variance (R Core Team, 2021). We used the "leveneTest" function from the "car" package to perform median-centered Levene's tests for homogeneity of variance (Fox & Weisberg, 2019). The "heatmap" function from the "stats" package was used to create heatmaps (R Core Team, 2021). We used the "lme4" package in R to create separate linear mixed models to assess the effects of aggression, site, and colony genetic strain on the total dry weight, total nutrients, and mass-corrected nutrients of each sample (Bates, 2015). Square-root and log+1 transformations were used as needed to normalize the data (noted in RESULTS as appropriate). Our initial models contained a single fixed effect (either aggression, site, or genetic strain) and colony identity as a random effect. We additionally created one model that included aggression, genetic strain, and the interaction between aggression and genetic strain as fixed effects and colony ID as a random effect. We used the DHARMa package to assess model diagnostics, which included a QQ plot, KS test, dispersion test, outlier test, group-level uniformity test, and Levene test (Hartig, 2022). We used the "car" package to run Type II ANOVAs to assess significance values for each model (Fox & Weisberg, 2019). Figures were created using the "ggplot2," "ggpubr," "cowplot," and "viridis" packages (Garnier et al., 2021; Kassambara, 2020; Whickam, 2016; Wilke, 2020). To run a Principal Components Analysis (PCA) on both total- and mass-corrected protein, lipid, and carbohydrate values, we used the prcomp() function in the "stats" package (R Core Team, 2021). The "ggbiplot" package was used to create visualizations of the PCA (Vu, 2011).

## 2.3 Results

### 2.3.1 General observations

We measured the amounts of proteins, lipids, and carbohydrates in 467 naturally occurring samples of worker jelly across 18 different colonies, collected from larvae that were age-matched to 2 days old (24-48 hours post-hatching). Our samples had an average dry weight of 1.24 ± 0.63 mg (mean ± SD) with 0.82 ± 0.50 mg of protein, 0.05 ± 0.03 mg of lipids, and 0.10 ± 0.06 mg of carbohydrates, with 0.26 ± 0.34 mg of other matter not accounted for by these three macronutrients (e.g. nondigestible material, micronutrients, minerals, etc.). These measurements give an average ratio of approximately 16:1:2:5 for proteins : lipids : carbohydrates : other matter (as percentages: 66.6% proteins, 4.4% lipids, 7.9% carbohydrates, and 21.1% other matter) for the worker jelly of these two-day-old larvae. Table 2.1 shows how these measurements (converted to percentage of total dry weight) compare with other studies that have measured worker jelly composition. Though there is considerable variation in worker jelly nutrient composition among studies, our measurements are within the ranges reported by other studies and study yielded results in close agreement to Wang et al. (2015), the most recent study that examined a similar-aged cohort of larvae. Differences among studies could be due to variation in methodology (as many of the studies are more than 50 years old and used very different protocols) or could represent true variation in worker jelly composition within or among larval age cohorts.

Table 2.1: Comparison of different studies examining the nutritional content of worker jelly. Proteins, lipids, and carbohydrates are reported as a percentage of the dry weight (± standard deviation, where reported). “Other” indicates the percentage of dry weight not accounted for by the three macronutrients (e.g. micronutrients, nondigestible material, etc.). NR=Not reported by the study.

| Study | Larval Age (hours post-hatching) | Sample Size (# samples; # colonies) | Proteins | Lipids | Carbo-hydrates | Other | Total Dry Weight (mg ± S.D.) |
|---|---|---|---|---|---|---|---|
| Planta, 1889 | 0-96 hrs. | 2000; NR | 53.4 % | 8.4% | 18.1% | 20.1% | 1.7mg |
| Kohler, 1922 | 0-96 hrs. | NR; NR | NR | 23.3% | 15.7% | NR | NR |
| Haydak, 1943 | 0-48 hrs. | NR | 78.3% | 17.7% | NR | NR | NR |
| Shuel & Dixon, 1959 | 0-30 hrs. | 3-6; 2 | 49.2 ± 2.3% | 5.2 ± 1.1% | 12.0 ± 3.2% | 33.6% | NR |
| Brouwers et al., 1987 | 0-84 hrs. | 2-15; 3 | 40-65% | ~6-10% | 12-20% | 5-42% | NR |
| Kunert and Crailsheim, 1988 | 0-96 hrs. | NR | 42-50% | NR | 9-19% | NR | NR |
| Wang et al., 2015 | 24-48 hrs. | 100; 5 | 50.7 ± 0.5% | NR | 9.6 ± 0.5% | NR | NR |
| **Present Study** | **24-48 hrs.** | **467; 18** | **66.6 ± 40.1%** | **4.4 ± 2.1%** | **7.9 ± 4.8%** | **21.1 ± 27.1%** | **1.24 ± 0.63 mg** |

We assessed the relationship of the three macronutrients within each sample and whether these relationships differed by aggression level using a Principal Components Analysis (PCA). For this analysis, we used both absolute (i.e. total) and relative (i.e. mass-corrected) measurements of proteins, lipids, and carbohydrates (Fig. 2.1). For absolute quantities, our first component (PC1) explained 75.6% of the total variance. Our second component (PC2) explained 15.9% of the variance for a cumulative 91.5% of the variance explained by our first two components. PC1 was largely explained by proteins while PC2 weighted lipids and carbohydrates opposite of each other. There was very little separation between aggression categories within this analysis, although high aggression colonies

showed greater spread along PC1 (heavily driven by protein) than low aggression colonies (Fig. 2.1A). For relative quantities, our first component (PC1) explained 59.4% of the total variance while our second component (PC2) explained 29.0% for a cumulative of 88.5%. Similarly to absolute quantities, PC1 was largely explained by proteins while PC2 weighted lipids and carbohydrates opposite of each other (Fig. 2.1B). We note that in the relative PCA, proteins and lipids were weighted much more closely together than carbohydrates. This may be due to the fact that honey bee proteins and lipids both come nearly entirely from pollen grains, so these two nutrients are strongly interrelated, while carbohydrates come somewhat from pollen but also heavily from nectar and honey (Brodschneider & Crailsheim, 2010). There was once again very little separation between high and low aggression colonies along these axes (Fig. 2.1B).

Figure 2.1

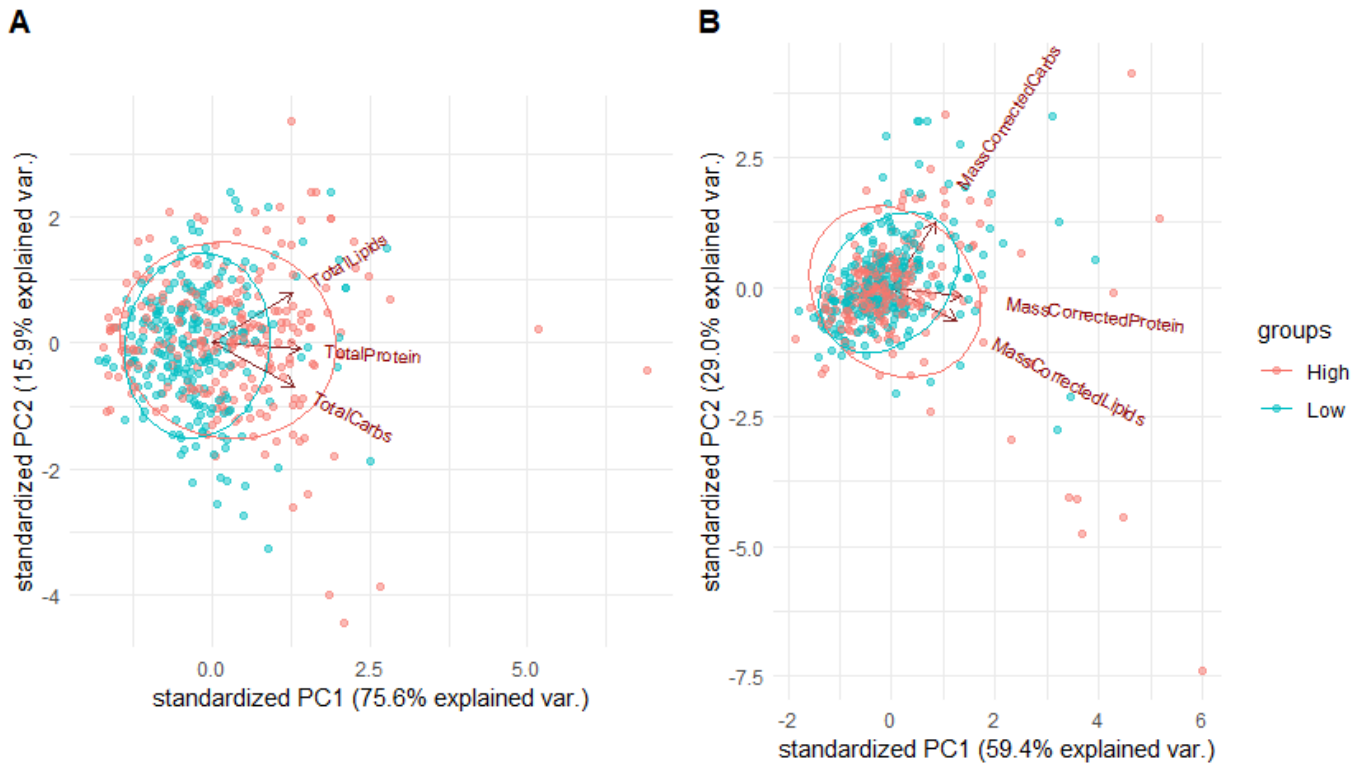



Figure 2.1: Aggression is not predictive of the total or relative nutrient contents of honey bee worker jelly. Principal Components Analysis (PCA) of A) total, and B) mass-corrected (fractional/relative) proteins, lipids, and carbohydrates with ellipses showing colony aggression level (high vs. low). Note high degree of overlap between categories, with slightly more variation in high-aggression colonies along PC1 of A). PC1 of both figures is largely driven by protein measurements, while PC2 loads lipids and carbohydrates opposite of one another.

### 2.3.2 Sample variability

We found large variation among our global sample set, with higher standard deviations among samples than the two previous studies that reported standard deviation (Shuel & Dixon, 1959; Wang, Ma, et al., 2016). Most previous studies used very small sample sizes, however, often fewer than 20 total samples from 5 or fewer colonies (see Table 2.1). Thus, these studies were not designed to detect natural variation, particularly among colonies. We found that there was considerable variation among colonies in the

total mass of each nutrient as well as relative nutrients (Fig. 2.2). Among-colony variation was higher than within-colony variation for total dry weight and total nutrients as assessed by Analysis of Variance (ANOVA: total dry weight square-root transformed: $F_{17}=24.4$, $p<0.0001$; total proteins square-root-transformed: $F_{17}=26.1$, $p<0.0001$; total lipids square-root-transformed: $F_{17}=17.3$, $p<0.0001$; total carbohydrates square-root transformed: $F_{17}=20.1$, $p<0.0001$). Mass-corrected nutrients were similarly significant. The existence of a significant colony effect suggests that sample variation in our global dataset is largely attributable to among-colony rather than within-colony variation. Total proteins showed the highest F-statistic (i.e. highest among-colony variance relative to within-colony variance) and total lipids showed the lowest F-statistic (i.e. still greater among-colony variance than within-colony variance, but more within-colony variance compared to the other nutrients). Thus, the large variation among samples appears to reflect consistent colony-level differences in worker jelly nutritional profiles.

Figure 2.2

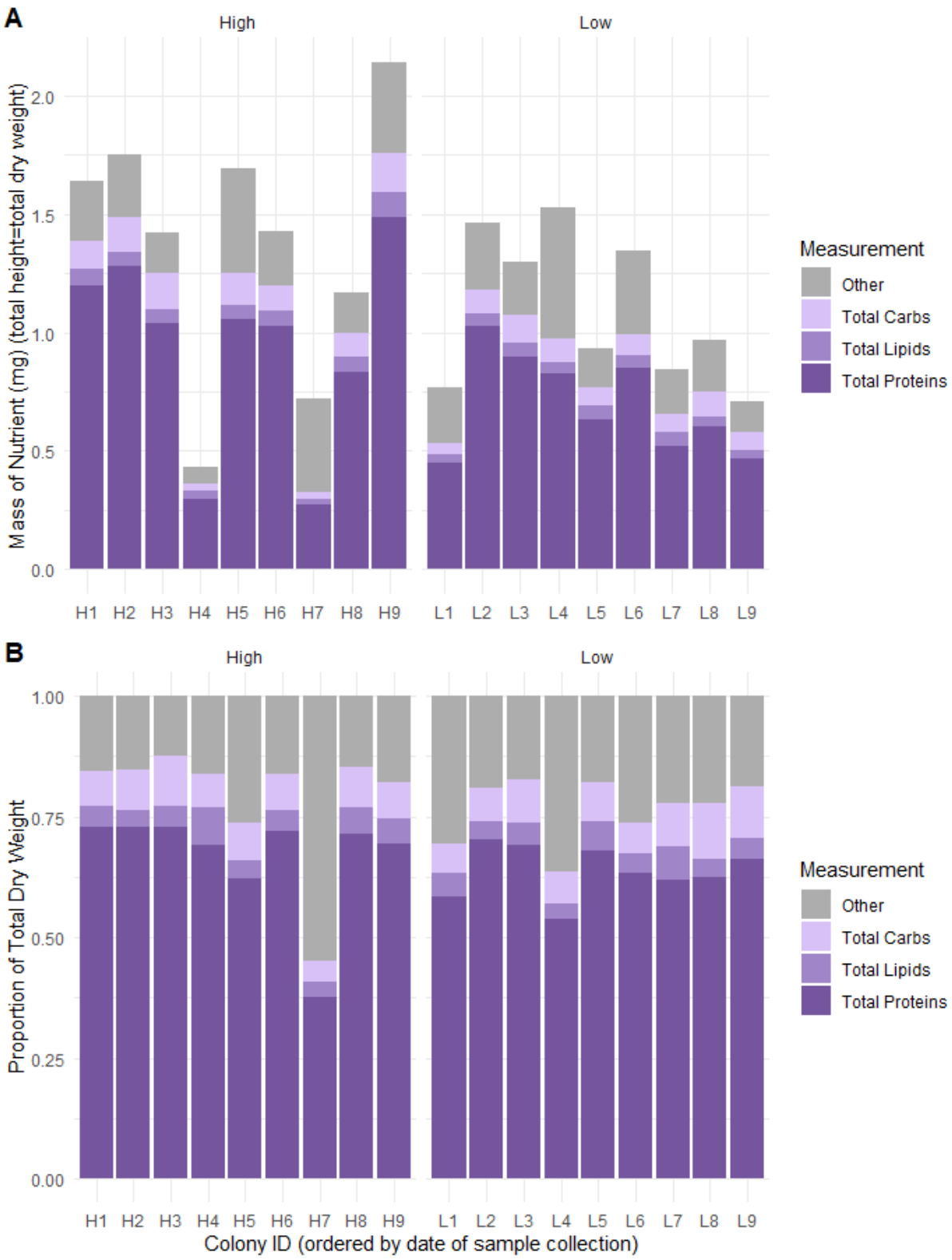

A
High
Low
Mass of Nutrient (mg) (total height=total dry weight)
2.0
1.5
1.0
0.5
0.0
H1 H2 H3 H4 H5 H6 H7 H8 H9
L1 L2 L3 L4 L5 L6 L7 L8 L9
Measurement
Other
Total Carbs
Total Lipids
Total Proteins
B
High
Low
Proportion of Total Dry Weight
1.00
0.75
0.50
0.25
0.00
H1 H2 H3 H4 H5 H6 H7 H8 H9
L1 L2 L3 L4 L5 L6 L7 L8 L9
Colony ID (ordered by date of sample collection)
Measurement
Other
Total Carbs
Total Lipids
Total Proteins

Figure 2.2: Colonies varied widely in both the total amount of each nutrient as well as the relative amounts of each nutrient. Colonies are arranged along the x-axis in the order in which the samples were collected (see Appendix 1, Table A1S1 for sample collection dates). High-aggression colonies are clustered on the left and low-aggression colonies are clustered on the right. A) Mean mass of each nutrient in mg; proteins on bottom in dark purple, lipids above in mid-purple, carbohydrates above in light purple, all other mass above in gray. The total height of the bar represents the mean total dry weight of the samples. B) Relative amounts of each nutrient as a proportion of the total dry mass of the sample; similar color scheme to A).

We additionally performed Levene's test for homogeneity of variance and found that total proteins showed significant non-homogeneity of variance among colonies, meaning that some colonies showed significantly higher within-colony variation than others. (Levene's test: total proteins square-root transformed: $F_{17}=2.76$, $p=0.0002$). No other measure of total nutrition was significant for Levene's test, meaning that colonies tended to have relatively similar within-colony variance for total dry weight, total lipids, and total carbohydrates. (Levene's test: total dry weight square-root transformed: $F_{17}=0.88$, $p=0.60$; total lipids square-root-transformed: $F_{17}=1.36$, $p=0.15$; total carbohydrates square-root transformed: $F_{17}=0.91$, $p=0.56$).

Expanding upon the above description of the observed among-colony variance, we note several unusual colonies and samples in our data. For example, the colony with the highest average total dry weight per sample (H9) is nearly five times higher than the colony with the lowest average total dry weight (H4). Additionally, as can be seen in Figure 2.2B, one colony (H7) had a significantly higher proportion of mass in the "other" category—that is, mass that is not accounted for by proteins, lipids, or carbohydrates. This phenomenon would likely not be due to technical error in the sample collection or measurement process, as all of H7's samples were collected and weighed at the same time

as another colony, L7, which showed typical amounts of "other" mass, and then all samples were randomized between plates during the colorimetric assays. We do not have a definitive explanation for why this occurred in only one colony or what comprises the unexplained "other" mass.

We visually assessed the relationship between the mean dry weight per sample and the within-colony variance in the mass of each macronutrient (Fig. 2.3). Colonies with lower mean dry weight per sample tended to have more variable within-colony carbohydrate mass (measured by coefficient of variation) and, to a lesser degree, more variable lipid mass, particularly in the lower 2/3 of the total dry weight range (i.e. the bottom 12 colonies). Proteins, however, did not show this pattern (Fig. 2.3). Though we collected all of our samples within a relatively short timeframe (23 days), we used simple linear regressions to verify that there was no relationship between the day of the collection (with the first collection day being day 1) and total dry weight, total proteins, total lipids, and total carbohydrates. We identified no significant relationship, reinforcing that there was no seasonal pattern in our data (Appendix 1, Fig A1S1; ANOVA: total dry weight: Wald $X^2_1$=0.76, p= 0.38; total proteins: Wald $X^2_1$=1.15, p= 0.28; total lipids: Wald $X^2_1$=0.008, p= 0.93; total carbohydrates: Wald $X^2_1$=0.27, p= 0.60). Our primary analysis pooled across all colonies, but results were similar when high- and low-aggression colonies were assessed separately.

Figure 2.3

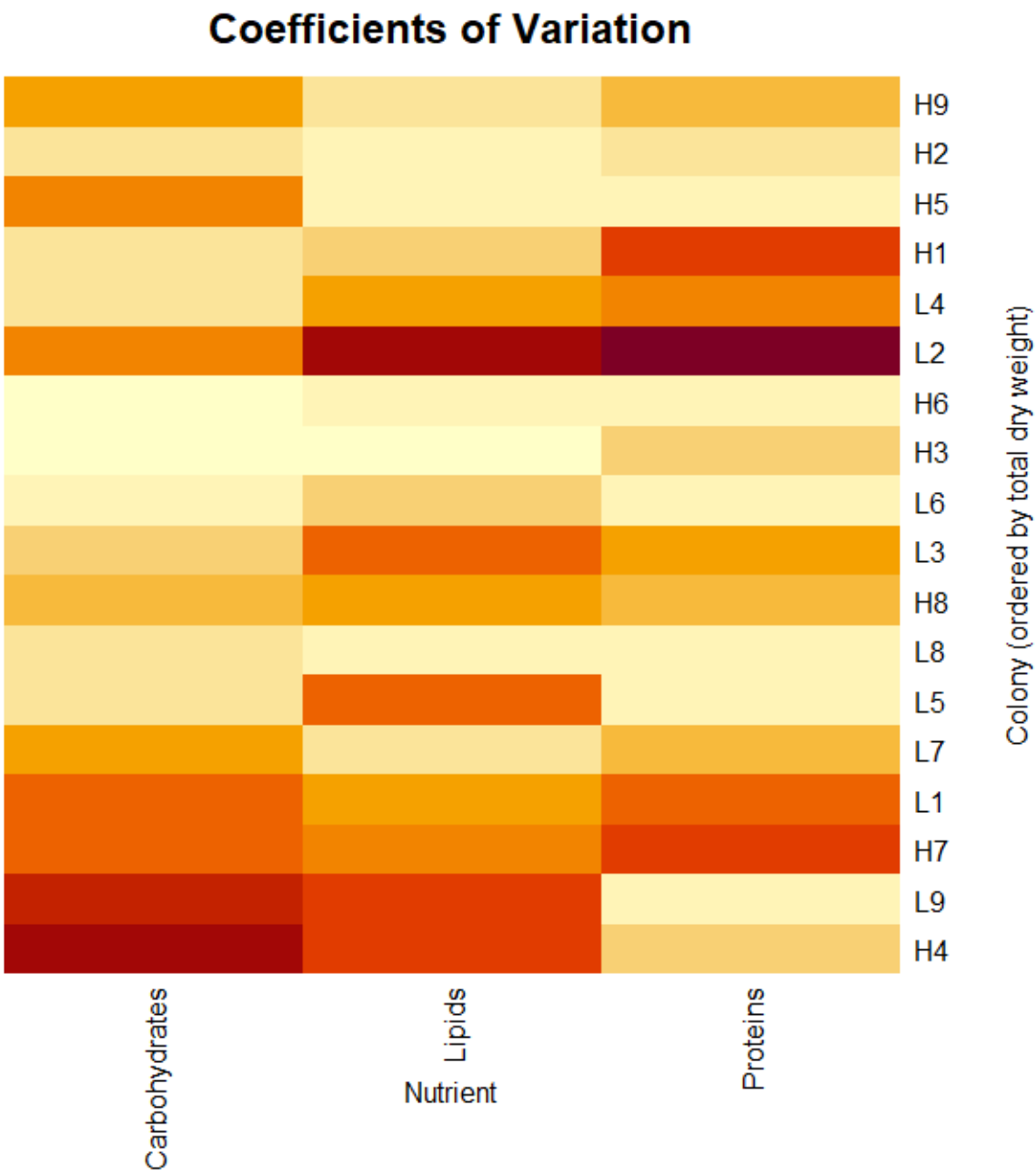

Coefficients of Variation
H9
H2
H5
H1
L4
L2
H6
H3
L6
L3
H8
L8
L5
L7
L1
H7
L9
H4
Colony (ordered by total dry weight)
Carbohydrates
Lipids
Proteins
Nutrient

Figure 2.3: Heatmap of coefficients of variation for total mass of carbohydrates, lipids, and proteins in each colony. Colonies are ordered vertically by increasing dry weight, with the lowest-weight sample at the bottom and the highest-weight sample at the top. Darker shades represent higher coefficients of variation. Worker jelly shows higher within-colony variation in carbohydrate (and to a lesser extent, lipid) content as dry weight decreases, particularly within the 12 lightest samples (the bottom 2/3 of the graph, boxed region). Proteins do not show a relationship between within-colony sample variation and dry weight.

### 2.3.3 Nutritional content of worker jelly is not associated with colony-level aggression.

To assess the relationship between colony aggression and worker jelly nutritional content, we first analyzed aggression as a binomial variable (low versus high aggression, see METHODS). All nutrients were analyzed with linear mixed models that contained Colony ID as a random effect (see "Statistical Analyses"). We found no association between colony aggression level and either total nutrients or mass-corrected nutrients (the total amount of one nutrient divided by the dry weight of the sample, a way to assess the ratio of nutrients within each sample; ANOVA: total dry weight log-transformed: Wald $X^2_1$=1.3, p= 0.25; total proteins log-transformed: Wald $X^2_1$=1.9, p= 0.17; total lipids square-root-transformed: Wald $X^2_1$=1.7, p= 0.19; total carbohydrates log-transformed: Wald $X^2_1$=1.2, p= 0.27; mass-corrected nutrients gave findings that were similarly non-significant). For the total dry weight, high aggression colonies had a mean of 1.4 ± 0.7 mg while low aggression colonies had a mean of 1.1 ± 0.5 mg. For the total proteins, high aggression colonies had a mean of 0.9 ± 0.6 mg while low aggression colonies had a mean of 0.7 ± 0.4 mg. For the total lipids, high aggression colonies had a mean of 0.06 ± 0.03 mg while low aggression colonies had a mean of 0.05 ± 0.02 mg. For the total carbohydrates, high aggression colonies had a mean of 0.1 ± 0.07 mg while low aggression colonies had a mean of 0.09 ± 0.05 mg. Results are displayed in Figure 2.4. Our finding

that neither total nor mass-corrected nutrients were associated with colony aggression are in accordance with our findings in the PCA analysis (see “General Observations”), where we saw very little separation by aggression level for either metric (Fig. 2.1).

Figure 2.4

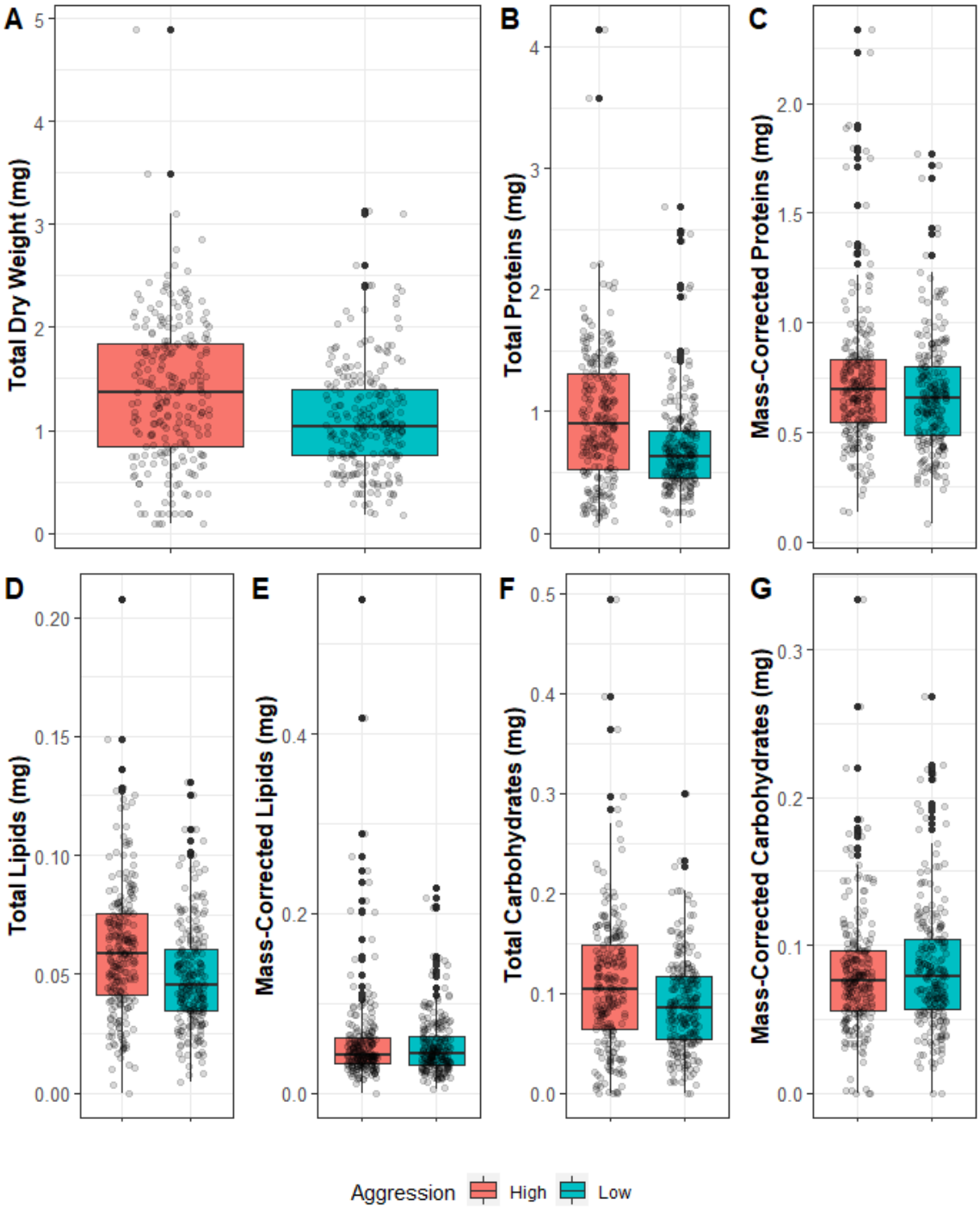

A
Total Dry Weight (mg)
B
Total Proteins (mg)
C
Mass-Corrected Proteins (mg)
D
Total Lipids (mg)
E
Mass-Corrected Lipids (mg)
F
Total Carbohydrates (mg)
G
Mass-Corrected Carbohydrates (mg)
Aggression
High
Low

Figure 2.4: Aggression level does not significantly explain variation in our measures of total food quantity, total mass of each nutrient type, or relative (mass-corrected) nutrients of each type. Boxplots of A) total dry weight , B) total proteins, C) mass-corrected proteins, D) total lipids, E) mass-corrected lipids, F) total carbohydrates, and G) mass-corrected carbohydrates of worker jelly samples from high- (red) and low- (blue) aggression colonies. All comparisons are statistically nonsignificant based on linear mixed models.

We additionally assessed the relationship between aggression and worker jelly content treating aggression as a ranked variable rather than a categorical variable. Each colony was assigned an aggression rank (1-18) based on the raw aggression score calculated in the colony-level aggression assays (see METHODS). Visually, there appears to be a weak positive correlation between most nutrient masses and aggression ranks (Fig. 2.5). However, we found no significant association between aggression rank and nutritional content using linear mixed models for each nutritional component with aggression rank (1-18) as a fixed effect and colony ID as a random effect (ANOVA: total dry weight: Wald $X^2_1$=2.4, p = 0.12; total proteins square-root transformed: Wald $X^2_1$=2.6, p = 0.11; total lipids square-root transformed: Wald $X^2_1$=1.8, p = 0.18; total carbohydrates: Wald $X^2_1$=1.9, p = 0.16). Results were similar when we ran models separately for high- and low-aggression colonies (Appendix 1, Fig A1S2), and when we treated aggression score as a continuous variable (see METHODS; Appendix 1, Fig. A1S3).

Figure 2.5

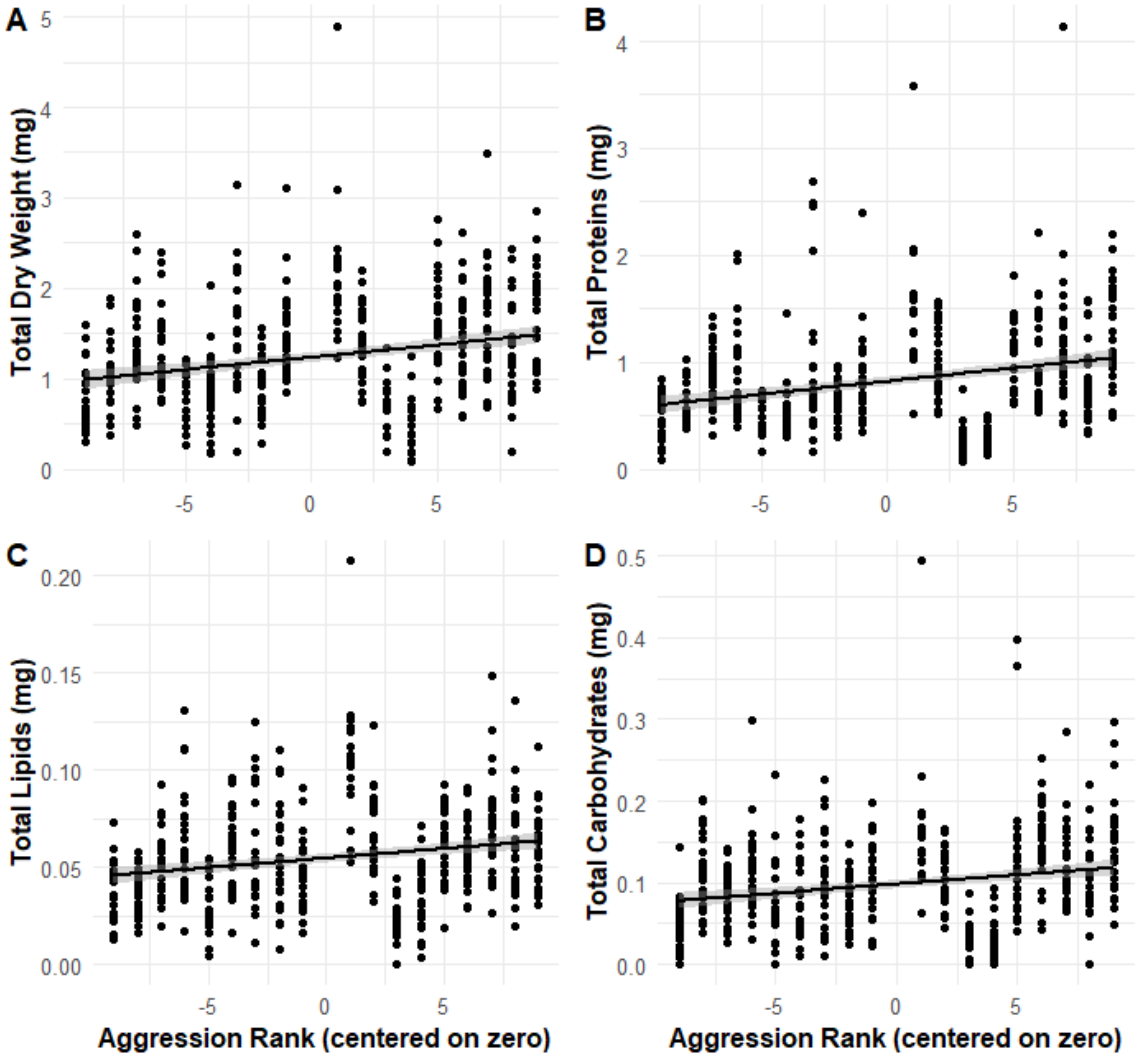



Figure 2.5: Ranked aggression score is not predictive of worker jelly nutritional profiles. Scatterplots of aggression ranks (centered around zero, lower aggression ranks to the left) versus A) total dry weight, B) total proteins, C) total lipids, and D) total carbohydrates of honey bee worker jelly samples.

Finally, we assessed how variance differed between high- and low-aggression colonies. Similar to the whole-data analyses described above, we used Analysis of Variance tests for each nutritional component within aggression level to test if aggression was associated with a change in within- versus among-colony variance. We found in all cases that among-colony variance was greater than within-colony variance, but that this

difference was vastly higher in high-aggression colonies; that is to say, high-aggression colonies were more variable on an among-colony basis, so they had much higher F-statistic values than low-aggression colonies. For example, the ratio of among- to within- colony variance for carbohydrates was 33.5 in high-aggression colonies while it was only 7.5 in low-aggression colonies. (ANOVA: total dry weight square-root transformed: high: $F_1$ =33.6, p <0.0001; low: $F_1$ =13.5, p <0.0001; total proteins square-root-transformed: high: $F_1$ =38.1, p <0.0001; low: $F_1$ =10.4, p <0.0001; total lipids square-root-transformed: high: $F_1$ =28.4, p <0.0001; low: $F_1$ =6.5, p <0.0001; total carbohydrates square-root transformed: high: $F_1$ =33.5, p <0.0001; low: $F_1$ =7.5, p <0.0001). High-aggression colonies also tended to have slightly higher within-colony variances in total dry weight and total protein compared to low-aggression colonies, but these differences were not statistically significant (ANOVA: variance of total dry weight: $F_1$ =1.51, p =0.24; variance of total proteins: $F_1$ =0.58, p =0.46; variance of total lipids: $F_1$ =0.02, p =0.88; variance of total carbohydrates: $F_1$ =1.14, p =0.30).

### 2.3.4 Alternative hypotheses for explaining variation in worker jelly nutritional content among colonies

In addition to aggression, we examined alternative factors that might drive variation in worker jelly content. The first hypothesis we examined was that location might influence variation in worker jelly nutritional content, because floral resources, and therefore pollen nutrient composition, can vary substantially among locations (Alburaki et al., 2018; Malagnini et al., 2022; Odoux et al., 2012). We predicted that worker jelly composition would be more similar among colonies in similar locations. All three sites were on relatively similar land use type, namely a mix of cropland, pastureland, and natural areas.

Two sites (“Alpha” and “Beta”) were approximately one mile apart. As honey bees prefer to forage close to the colony when possible (often under half a mile in ideal conditions), colonies at sites “Alpha” and “Beta” would likely have some overlapping foraging territory but would also have access to some unique resources (Beekman & Ratnieks, 2000; Danner et al., 2017; Hagler et al., 2011; Seeley, 1994). Conversely, site “Gamma” was nine miles away from the other two sites and therefore would comprise a completely unique set of available nutritional resources. We analyzed nutrients with linear mixed models with site identity as a fixed effect and Colony ID as a random effect. We found no significant differences in total dry weight, total proteins, total lipids, or total carbohydrates as a function of site identity (Fig. 2.6; ANOVA: total dry weight log-transformed: Wald $X^2_2$=2.9, p = 0.24; total proteins square root-transformed: Wald $X^2_2$=2.4, p = 0.30; total lipids square-root-transformed: Wald $X^2_2$=0.3, p = 0.85; total carbohydrates: Wald $X^2_2$=2.8, p = 0.25). Results were similar for mass-corrected proteins, lipids, and carbohydrates.

Figure 2.6

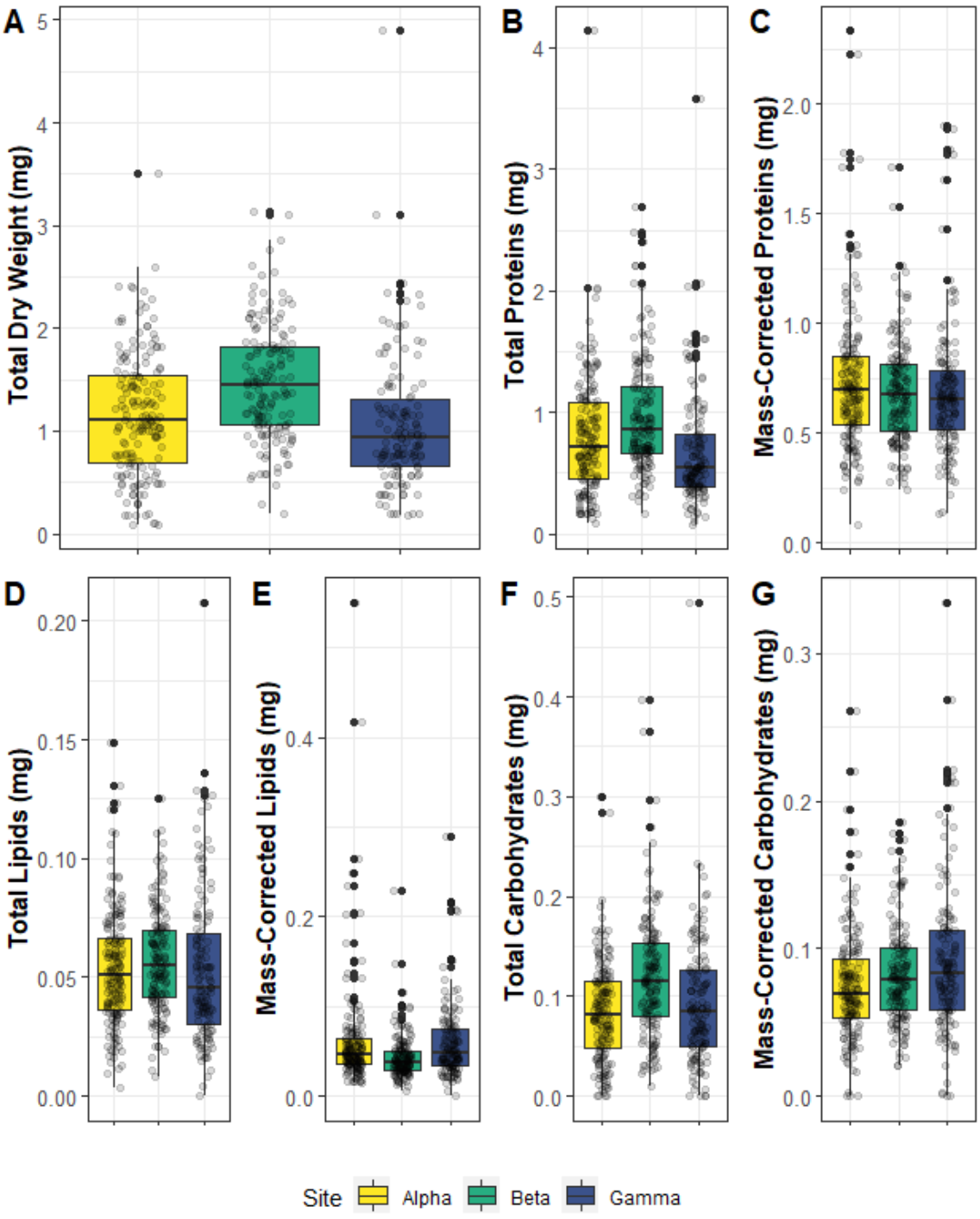

A
Total Dry Weight (mg)
0
1
2
3
4
5
B
Total Proteins (mg)
0
1
2
3
4
C
Mass-Corrected Proteins (mg)
0.0
0.5
1.0
1.5
2.0
D
Total Lipids (mg)
0.00
0.05
0.10
0.15
0.20
E
Mass-Corrected Lipids (mg)
0.0
0.2
0.4
F
Total Carbohydrates (mg)
0.0
0.1
0.2
0.3
0.4
0.5
G
Mass-Corrected Carbohydrates (mg)
0.0
0.1
0.2
0.3
Site
Alpha
Beta
Gamma

Figure 2.6: Site was not predictive of worker jelly nutritional content. Samples of worker jelly were collected from colonies in three yards: "Alpha" and "Beta" were approximately 1 mile apart, while "Gamma" was approximately 9 miles away from the other two sites. Linear mixed models of each nutrient with site as a fixed effect and colony ID as a random effect all showed no significant differences. Boxplots of A) total dry weight , B) total proteins, C) mass-corrected proteins, D) total lipids, E) mass-corrected lipids, F) total carbohydrates, and G) mass-corrected carbohydrates of worker jelly samples at these three sites. Appendix 1, Figure A1S4 shows a similar graph, but with the individual points colored based on colony aggression level.

We additionally examined whether the genetic strain of the colony might predict variation in worker jelly. These analyses included 411 samples from 16 colonies representing three genetic strains, "Italian," "Russian Hybrid," and "Wild Stock" (see "Methods"; two colonies were excluded prior to analysis because they had unique genetic backgrounds not represented by any other colonies in the experiment). We did not detect statistically significant variation in worker jelly as a function of genetic strain (Fig. 2.7; LMM with Colony ID as a random effect; ANOVA: total dry weight log-transformed: Wald $X^2_2$=5.6, p= 0.06; total proteins square root-transformed: Wald $X^2_2$=0.9, p = 0.64; total lipids square-root-transformed: Wald $X^2_2$=1.3, p = 0.52; total carbohydrates square root-transformed: Wald $X^2_2$=2.5, p= 0.29). Results were similar for mass-corrected values. Genetic strain is correlated with aggression in some contexts, although this association is not ubiquitous and is often complex (Alaux et al., 2009; Harpur et al., 2020; Locke, 2016). In our data, both Russian Hybrid and Wild Stock strains were represented by colonies of both high and low aggression level. All of our Italian colonies, however, were high aggression (See Appendix 1, Table A1S1). We therefore ran an additional model that included both genetic strain and aggression as well as an interaction term between aggression and genetic strain, but this model yielded similar results (LMM with Colony ID as a random effect; ANOVA: total dry weight log-transformed: strain: Wald $X^2_2$=1.5, p=

0.48; aggression: Wald $X^2_1$=2.4, p= 0.12; strain*aggression interaction: Wald $X^2_1$=0.2, p= 0.68; total proteins square root-transformed: strain: Wald $X^2_2$=2.1, p= 0.36; aggression: Wald $X^2_1$=1.1, p= 0.29; strain*aggression interaction: Wald $X^2_1$=0.005, p= 0.94; total lipids square-root-transformed: strain: Wald $X^2_2$=0.1, p= 0.94; aggression: Wald $X^2_1$=1.4, p= 0.24; strain*aggression interaction: Wald $X^2_1$=0.005, p= 0.94; total carbohydrates square root-transformed: strain: Wald $X^2_2$=3.1, p= 0.21; aggression: Wald $X^2_1$=0.002, p= 0.97; strain*aggression interaction: Wald $X^2_1$=0.3, p= 0.59). Versions of Figures 2.6 and 2.7 that feature dots colored by aggression level can be found in Appendix 1 (Appendix 1, Figs. A1S4 and A1S5).

Figure 2.7

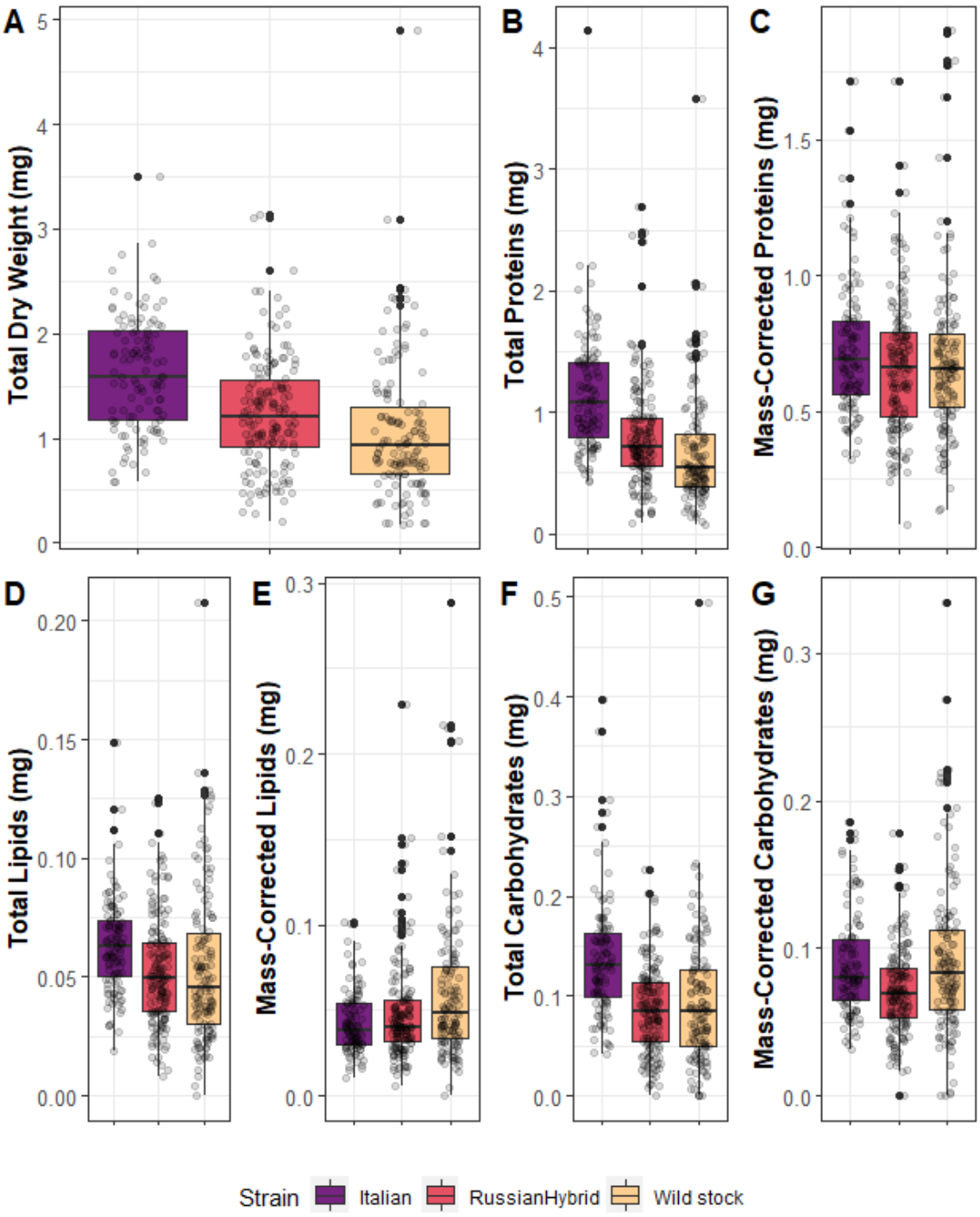

A
Total Dry Weight (mg)
B
Total Proteins (mg)
C
Mass-Corrected Proteins (mg)
D
Total Lipids (mg)
E
Mass-Corrected Lipids (mg)
F
Total Carbohydrates (mg)
G
Mass-Corrected Carbohydrates (mg)
Strain
Italian
RussianHybrid
Wild stock

Figure 2.7: Genetic strain of the colony was not predictive of worker jelly nutritional content. Some colonies were established as packages with strains advertised as "Italian" (4 colonies) and "Russian Hybrid" (6 colonies) at the beginning of the season. All but one of the package colonies still contained the original foundress queen, the other being a direct descendant of the foundress. Other colonies were of mixed local genetic stock ("Wild Stock," 6 colonies). Linear mixed models of each nutrient with strain as a fixed effect and colony ID as a random effect all showed no significant differences. Boxplots of A) total dry weight, B) total proteins, C) mass-corrected proteins, D) total lipids, E) mass-corrected lipids, F) total carbohydrates, and G) mass-corrected carbohydrates in the worker jelly from colonies of these three genetic strains. Appendix 1, Figure A1S5 shows a similar graph, but with the individual points colored based on colony aggression level.

## 2.4 Discussion

We measured the amount of total food and the amounts of proteins, lipids, and carbohydrates in naturally occurring samples of worker jelly from age-matched honey bee larvae. We hypothesized that samples of honey bee worker jelly would show low variation in macronutrients, particular proteins, due to the multiple layers of filtering and processing of the nutritional components between being collected on a flower and being eaten by the larva. Contrary to our predictions, we found substantial within- and among-colony variation in total dry weight, proteins, lipids, and carbohydrates in worker jelly fed to age-matched honey bee larvae. This variation echoes the variation that has been found in other animals that create nutritional secretions to feed their young, despite these other instances often involving one carer instead of the combined efforts of many workers as seen in honey bees (Amigo & Fontecha, 2011; Laben, 1963; Michaelsen et al., 1990). Even with relatively high within-colony variance, we found that among-colony variance was significantly higher. These differences likely represent consistent colony-level differences in the nutritional makeup of worker jelly.

Particularly surprising in light of our hypotheses, we found that proteins showed the highest among-colony variance and additionally displayed non-homogenous within-

colony variance (i.e. some colonies had significantly greater within-colony variance than others). Meanwhile, lipids showed the highest relative within-colony variance compared to the other nutrients, potentially due to their low overall concentrations. Another possibility for the high within-colony variation in lipids is contamination by lipid compounds found in the wax of the honeycomb cells the worker jelly was extracted from, although all cells were treated similarly during extraction in terms of the timing and amount of water used as well as the vigorousness of homogenization (i.e. pipetting) during extraction. That proteins and lipids were the two components that showed the most variation (among-colony for proteins, within-colony for lipids) is also surprising in the context of recent research on pollen preferences. Many studies of honey bee nutrition at the colony level (as well as studies on other social bees) have focused on the protein to lipid ratios of the pollen brought into the colony by the foragers (Vaudo et al., 2016; Vaudo et al., 2020). This focus is pertinent to honey bee foraging ecology, given that proteins and lipids largely come from the same source (pollen) whereas carbohydrates come somewhat from pollen but largely from nectar and honey (Brodschneider & Crailsheim, 2010). And indeed, a recent study found that protein and lipid content of "bee bread" (stored and partially processed pollen within the colony that reflects longer-term nutritional reserves) was associated with floral diversity, but that carbohydrates were not (Donkersley et al., 2017). The type of pollen ingested has also been shown to affect components of lipid and amino acid metabolism in particular (Chang et al., 2023). Our results reflect these dynamics somewhat—for example, we found in our principal component analysis of mass-corrected nutrients that proteins and lipids were weighted more closely to one another than to carbohydrates. But one could also expect that proteins and lipids would be more

nutritionally constrained (and therefore less variable) because of these foraging realities while carbohydrates would be freer to vary. Our findings that the highest among-colony variation was in proteins and the highest within-colony variation was in lipids contradict this possibility. Recent work shows that nurse bees, unlike foragers, do not seem to show nutrition-based preferences for pollen when they consume it, which could help explain the disconnect between forager input and nurse output (Corby-Harris et al., 2018).

Given the detrimental effects of early life malnutrition, why do we see such striking variation in the nutritional properties of worker jelly? One possibility is that, once a minimum nutritional threshold is met, any further variation is less important for health and fitness consequences. Selective pressure on these organisms would drive them to meet the minimum but variation would be able to proliferate once that threshold was reached. This is the hypothesis that was put forward by Langer in 1929 to explain differences seen in worker jelly nutritional content between several different studies conducted in the late 1800s-early 1900s (Langer, 1929). While possible, studies in other taxa have found that substantial health effects can arise from relatively minor differences in early-life diet (Doherty et al., 2018). The nutritional elements where we saw the most among-colony variation were total dry weight and total proteins, and these are two elements that have been well documented to have detrimental health effects if not properly supplied to larvae (Brodschneider & Crailsheim, 2010).

Another possibility for why there is inter-colony variation is that colonies are somehow limited in what nutritional profile they are able to provide, although our analyses failed to uncover what factors could be associated with such a limitation. Such limitations could occur at multiple levels, either by limits on what nutrients the colony is able to bring

in or by limits on what an individual nurse is able to synthesize. Variation was not well explained by colony aggression level, site of the colony, or colony genetic strain despite the substantial among-colony variation in worker jelly nutritional content we observed. These dynamics could be complex—for example, while site is a feature that would likely be most impactful at the level of colony resource acquisition, genetic strain could affect either individual nurse physiology (i.e. synthesis of the worker jelly) or factors such as foraging preferences and ability. Aggression is correlated with foraging activity but is also associated with physiological differences in honey bees, so this feature could be associated with constraints at both levels (Chandrasekaran et al., 2015; Li-Byarlay et al., 2014; Rittschof et al., 2015; Rittschof et al., 2019; Rittschof et al., 2018; Wray et al., 2011). Among-colony variance was greater for high-aggression colonies than low-aggression colonies. This finding could be due to the fact that many factors can contribute to a colony's level of aggression, from genetics and colony size to weather and recent disturbances (Alaux et al., 2009; Rittschof, 2017; Schneider & McNally, 1992; Southwick & Moritz, 1987). If our high-aggression colonies were aggressive for different reasons, then some of these latent variables could be contributing independently to variation in the worker jelly. This result could also be looked at in another light: that within-colony variance was relatively higher in low-aggression colonies than high-aggression colonies. Low aggression has been associated with negative health outcomes in honey bees (Rittschof et al., 2019). Perhaps the greater relative contribution of within-colony variance suggests that these low-aggression colonies struggle to hit nutritional targets as consistently as their high-aggression counterparts.

We chose to measure age-matched two-day-old worker larvae. This decision minimized the contribution of age-related variation to worker jelly nutritional content. Our study cannot say whether other larval stages would show more, less, or a similar amount of variation, however, or if factors such as aggression would play a bigger role in determining variation at other stages should it exist. We collected our samples within a short time period, and we did not observe any trend with time, precluding seasonal effects as an explanation for the among-colony variation seen in our study. Other factors that we did not measure could explain the high level of variation we detected. For example, colony size has far-reaching effects on overall colony health and foraging ability (Beekman et al., 2004). Though we only used colonies that were at a full, mature size at the time of our experiment, it is possible that subtler differences in the worker population could have contributed to our results. Other factors like disease, the age distribution of the nurses, the ages of larvae on adjacent frames, or the cumulative effects of multiple stressors could have caused these effects. And although the broad measure of colony genetic strain was not predictive, it is possible that more fine-scale genetic differences tied to individual queens or even individual workers' patrilines could have had an effect. Future studies could explicitly test these possibilities.

We did not measure individual or colony-level outcomes for the larvae that were raised on these diets. It is possible that even the large within- and among-colony variation we observed would not be enough to cause lethal or sublethal effects on the bees in these colonies. Based on previous work, though, we can make inferences about potential outcomes should any effects exist. For example, some of our colonies had much less total food than others (for example, colony H4; Fig. 2.2). Nurse bees in this colony could

potentially make up for less food per feeding by replenishing the food more frequently. If they do not do this, though, general underfeeding has been associated with a number of detrimental health outcomes. First, higher rates of developmental failure during the pupal stage as well as alterations to body weight and morphometrics have been seen in larvae that experience food deprivations (Gontarski, 1953; Jay, 1964). Furthermore, larvae that are subjected to insufficient nursing effort (by decreasing the number of nurses rather than removing food) show a ~25% decrease in adult lifespan and altered morphometrics relative to conditions with sufficient nursing effort (Daly et al., 1995; Eishchen et al., 1982). Conversely, Schilcher et al. recently found that minor underfeeding of larvae led to smaller adult body size but no further differences in physiological or behavioral outcomes (Schilcher, Hilsmann, Ankenbrand, et al., 2022). The maximum range of total food variation found in our study was greater than the experimental manipulation used in this work, though, making direct comparison difficult (with the "undernourished" condition being approximately 7% less food volume in their study, while our highest dry weight was more than five-fold greater than our lowest dry weight). In addition, we found the greatest among-colony variance in proteins in our study. Differences in protein type and amount in worker jelly have been shown to affect adult outcomes. It is common practice for in-vitro rearing studies of honey bees to use yeast in place of pollen as a protein source for the synthetic diet. Adults raised with these diets show relatively normal body weights and survival rates but have altered nursing and foraging behaviors in addition to having altered hormone profiles in adulthood (Aupinel et al., 2005; Schilcher, Hilsmann, Rauscher, et al., 2022). These results concur with other findings that have shown that pollen (the primary natural source of protein) deprivation in a full-colony setting can negatively affect the adult

behavior and physiology of workers raised in these colonies, demonstrating that nurses are not always able to fully compensate for adverse nutritional availability in natural settings. (Scofield & Mattila, 2015). Finally, different concentrations and ratios of carbohydrates in artificial in-vitro rearing diets have been shown to affect caste determination, with higher sugar content leading to more queen-like workers developing (Kaftanoglu et al., 2011).

This study has demonstrated that a critical component of early life, nutrition, shows high among-colony variation in naturally occurring honey bee worker jelly. This consistent variation exists similarly to the high variation seen in nutritional secretions in mammals, even though the provisioning of honey bee offspring is distributed among thousands of carers instead of one lactating parent. Our study took the larval perspective and examined the food in a cell available to a larva at a single point in time, which could include additions from multiple nurses. Future studies could seek to determine the level of individual variation from the perspective of the nurse bees by extracting worker jelly from individual nurses and comparing it with that of her colonymates. Honey bee larvae are able to achieve a remarkable amount of growth within the six days of larval development. They receive round-the-clock care provided by nurse bees, growing by over a hundred times their weight at hatching by the time they pupate, each larva being inspected by nurses upwards of 3,000 times per day (Rembold et al., 1980; Siefert et al., 2021). Previous theoretical and experimental work has suggested that parental care can buffer against environmental variability and risk on an evolutionary scale (de Zwaan et al., 2019). The role of within-species individual variation in these dynamics provides an exciting new avenue for studying the developmental, physiological, behavioral, and health consequences of the early life period.

# CHAPTER 3. RESPONSE TO COMPETING CONSPECIFIC CUES DEPENDS ON SOCIAL CONTEXT IN THE HONEY BEE (APIS MELLIFERA).

*Rebecca R. Westwick, Gavin P. Brackett, Cameron E. Brown, Bethany J. Ison, Clare C. Rittschof*

This chapter is currently under peer review at Animal Behavior. The first round of revisions has been submitted.

Animals exist in a world that is replete with sensory information. Not all of this sensory information is relevant to the organism at a given time, though. Understanding how animals are able to pick out "the signal from the noise" has been of interest to behaviour and neuroscience researchers for decades. This problem may be especially challenging when the conflicting sensory "noise" is also a conspecific signal, given that organisms often show heightened sensitivity to conspecific cues. We challenged nurse honey bees who were performing larval caretaking behaviours with honey bee alarm pheromone, a conspecific cue that they are able to detect but show low behavioural sensitivity to compared with other honey bee workers like guards and soldiers. We found that nurse bees that originated from high-aggression colonies decreased their larval caretaking behaviours in the presence of alarm pheromone, while nurses from low-aggression colonies did not show this change. Our work highlights the importance of considering social context when examining how organisms respond in the face of a sensory-rich world.

## 3.1 Introduction

Communication, the process of sending and receiving informative signals, is critical for social species. This is particularly true for species that live in large, complex

societies (Marler & Vandenbergh, 1979; O'Donnell & Bulova, 2007). Successful communication requires a receiver that is able to both detect and correctly process the relevant signal (Kaplan, 2014; Seyfarth & Cheney, 2003). For a receiver, this process can be difficult when competing information is present, a concept known in human systems as "the cocktail party problem" (Cherry, 1953). Competing stimuli can cause interference at multiple points along neurosensory pathways, from peripheral sensory systems (as with energetic masking, information overload, and olfactory receptor antagonism) to central processing (as with informational masking, distraction, and cross-modal interference) (Milinski, 1990; Oka et al., 2004; Rosa & Koper, 2018). Competing information is often a pervasive, relatively persistent feature of the environment the organism is in. For example, anthropogenic noise is detrimental to an enormous variety of organisms, impairing everything from intra-species communication to predator avoidance (Butler & Maruska, 2020; Chan et al., 2010; Kunc & Schmidt, 2019). Similarly, background odors (such as plant volatiles) create an "odorscape" that can alter behaviours that rely on olfaction such as foraging and mate finding (Conchou et al., 2019; Deisig et al., 2014; Schröder & Hilker, 2008). But competing information can also come from a more transient source, such as a sudden noise that causes a startle response or a cue from another animal (Elwood et al., 1998; Moorhouse et al., 1987). With so many places along the neurosensory trajectory for interference to occur, any stimulus that causes particularly strong activation of the nervous system has the potential to alter behaviour or impair communication. One such example can be found with conspecific cues.

Many animals show special sensitivity to conspecific information across multiple sensory modalities. (Braaten & Reynolds, 1999; Hattori et al., 2010; Kano & Call, 2014).

This special sensitivity can involve greater precision in discriminating between similar stimuli. This phenomenon has been especially well documented in olfaction, a modality with high potential for specificity where receivers are commonly (though not always) tuned narrowly to precise blends of pheromones (Buchinger & Li, 2020; Endler et al., 1993; Li et al., 1995). Special sensitivity can also manifest as lower response thresholds, where organisms are sometimes able to detect conspecific information at remarkably low signal strength (Kaissling & Priesner, 1970; Stengl, 2010). This special sensitivity comes about via multiple levels of sensory organization, from the tuning of olfactory receptors to neuronal organization that selectively amplifies conspecific pheromones (Sakurai et al., 2014; Tabuchi et al., 2013). Given this heightened sensitivity, conspecific signals could represent an especially potent source of conflicting information that could affect behavioural responses, but this idea remains untested.

Honey bees (*Apis mellifera*) provide a unique opportunity to study how different conspecific signals can interfere with each other, particularly in the context of olfactory signaling. Advanced eusociality in bees (e.g. honey bees, stingless bees) is associated with elaborated pheromone signaling (Fischman et al., 2011; Wittwer et al., 2017). Honey bees live in large, dense, enclosed nests where at least a dozen different pheromones can be in play at once (Bortolotti & Costa, 2014). Some of these pheromones are primer pheromones that are constantly present in the background and play out their effects over longer timescales, such as larval esters that suppress worker ovary development and have slow-acting effects on forager effort to collect different resources; other pheromones have acute releaser effects, and these are often more specific to particular in-hive tasks (reviewed in (Slessor et al., 2005). Individual worker bees temporarily specialize on these different tasks

at distinct times in their adult lives, but all tasks occur in the colony simultaneously (Seeley, 1995). This creates a system where individuals are exposed to a large suite of social signals but need to attend to only a subset of them at a given time. The prevailing model suggests that individuals show different sensitivity thresholds to cues that induce each task (Beshers & Fewell, 2001). Given the same level of a stimulus, some individuals will be more likely to respond than others despite similar perceptual abilities, allowing colonies to distribute labor demands amongst many thousands of individuals. Hormonal variation associated with behavioural specialization alters the probability that an individual worker will respond to a task-specific stimulus, allowing that individual to focus on task-specific social cues; as workers age and transition to new specializations, their hormonal milieu and stimulus thresholds shift in parallel (Robinson, 1987b). There is also evidence that perceptual abilities differ among specialists. For example, workers performing different jobs have different proteomic signatures in their antennae (Iovinella et al., 2018). This finding suggests that olfactory receptor protein abundance varies with task, potentially facilitating specialization. Overall, the conventional view is that behavioural specialists parse diverse cues in the nest and pay attention and respond primarily to task-relevant information.

Despite this conventional view, however, there is evidence that certain types of social information cross the lines of behavioural specialization and that workers pay attention to a greater range of information than previously appreciated. One example of such complexity is in the context of honey bee defensive aggression. Guards and soldiers are two types of defensive specialists that preferentially respond aggressively to threats to the colony (Breed et al., 2004). They stand near the entrance of the nest and perform characteristic attack behaviours in response to threats (Moore et al., 1987). Both specialist

types also emit and respond to a social cue, the honey bee "alarm pheromone," a blend of compounds in which isopentyl acetate (IPA) is a primary component (Boch et al., 1962; Boch & Shearer, 1971). The primary function of alarm pheromone is to recruit additional defensive specialists, especially soldiers, in response to an escalating or persistent threat (Breed et al., 2004). However, ample evidence shows that non-defensive specialists, including honey storers, individuals in the brood nest, returning foragers, and lab-reared young and middle-aged workers, also sting and/or respond to alarm pheromone in certain contexts (Allan et al., 1987; Breed et al., 1990; Burrell & Smith, 1994). This generalized response to alarm cues could suggest that these are particularly salient social cues for honey bee workers. Recent studies show that even pre-adult (larval and pupal) worker bees are sensitive to the level of defensiveness (and thus potentially the degree of alarm pheromone emission) displayed by their natal nestmates: individuals that develop in a relatively high-aggression colony show behavioural and physiological consequences in adulthood (Rittschof et al., 2015; Rittschof et al., 2019). As larvae largely lack sensory structures (Betts, 1923; Eichmütler & Schäfer, 1995), one explanation for this effect is that nurse bees (brood care specialists) alter their interactions with larvae in high aggression colonies, possibly through differential response to alarm pheromones released by nestmates. This result would be surprising however, as nurses are classically considered to be non-defensive brood care specialists (Johnson, 2008; Pearce et al., 2001). Our goal in the current study is to assess whether nurses do indeed pay attention to alarm cues, a response that may ultimately shape the phenotype of the developing larvae under their care.

Nurses check on and feed the larvae within the brood nest, responding in part to a putative "begging pheromone," e-β-ocimene, that is released by starving larvae and

provokes nurse visits (He et al., 2016). Nurses show relatively low behavioural responsiveness to alarm pheromone and are much less likely to behave aggressively in general compared to guards and soldiers (Collins, 1980; Pearce et al., 2001; Robinson, 1987a). However, this lowered responsiveness is not a matter of detection abilities, as assessed using electroantennogram assays (Robinson, 1987a). In the current study, we assess the possibility that alarm pheromone competes with larval olfactory cues to alter nursing behaviour. We test and compare individuals from relatively high and low aggression colonies to evaluate whether colony level variation in alarm cue sensitivity is reflected in nurse behaviour. Such a result would suggest a more complex system of cue integration than previously appreciated in the honey bee.

## 3.2 Methods

We used observation hives and video recordings to measure variation in nursing behaviour directed towards individual honeycomb cells in three treatment groups that differed in the quantity of begging cue: larvae, larvae supplemented with begging pheromone, and empty cells (a control). We further observed these behaviours with and without whole-colony exposure to an interfering social signal, alarm pheromone. We evaluated nurses from high and low aggression colonies (colonies that are more or less responsive to defense-inducing cues as described below) to determine whether colony response thresholds predict nursing behavior generally and/or the nurse behavioral response to alarm pheromone. A diagram of our experimental treatments can be found in Appendix 2 (Appendix 2, Fig. A2S1).

### 3.2.1 Honey bee sources

We performed experiments in Lexington, Kentucky, USA during July-October 2019 and 2020. The colonies from which we sourced the nurses and brood had mostly been installed as packages at the beginning of the season (strains advertised as “Italian” and “Russian Hybrid”). Remaining colonies were of mixed local genetic stock. All colonies were maintained according to standard management practices and parasite control measures as suggested by the Honey Bee Health Coalition. Only colonies that were at full, mature size and were healthy at the last check were used in the experiment (i.e. queenright, not showing any overt signs of disease, not undergoing active mite treatment).

### 3.2.2 Identifying high and low aggression colonies for nurse bee collection

Following Rittschof et al., 2015, we surveyed ~30 colonies for response to alarm pheromone, which is a measure of defensive aggression (Collins & Kubasek, 1982). Briefly, we photographed the landing board of each hive to measure the baseline activity level of a colony, which is the number of bees that could be seen on the landing board of the hive in the photograph. We then introduced a small piece of filter paper with 3uL of 1:10 isopentyl acetate (hereafter IPA, a primary component of the honey bee alarm pheromone; (Boch et al., 1962) in mineral oil, gave one minute for the bees to respond, and then took a second picture of the number of bees on the landing board. This amount of IPA is within the standard range for field aggression tests (Boch & Rothenbuhler, 1974; Collins & Kubasek, 1982; Collins et al., 1987). It is the estimated amount of IPA released by guard and soldier bees during a strong colony-level defensive response (Allan et al., 1987; Collins & Rothenbuhler, 1978). When IPA is placed at the entrance, bees emerge from the entrance

in response, typically congregating at the site of the filter paper, or crawling up the front of the hive. Because the bees rarely take flight, the second photo of the entrance captures the IPA response (Collins & Kubasek, 1982; Guzmán-Novoa et al., 2003). We calculated the colony's response score as the difference between the number of bees on the landing board and on the front of the hive after the IPA was placed and the baseline activity. We define an experimental "round" as being the nurses from a single colony tested over two days (see "*Nurse Behavior Assay and Recordings*"). For each pair of experimental rounds, we selected one colony with the highest and one colony with the lowest IPA response score to be the source colonies for nurse bees. No colony was used more than once as a source colony during the study. Overall, we included 6 colonies per aggression level (N=12 colonies total, three high and three low aggression colonies in 2019, and three high and three low in 2020). Trials were conducted within 2 weeks of an aggression assay, as colony aggression level can vary over the season (Pearce et al., 2001; Schneider & McNally, 1992) T. Napier, *unpublished data*).

### 3.2.3 Larval treatments and manipulation of begging pheromone

We generated three larval treatments that differed in the quantity of begging pheromone to assess variation in nurse bee behavior with and without interference from alarm pheromone. Honey bee larvae develop in individual honeycomb cells (one larva/cell). Our treatments included (*1*) a larva alone (unmanipulated), (*2*) a larva to which we added 10 uL 1:10 e-β-ocimene (hereafter EBO) in mineral oil, gently pipetted on the sides of the honeycomb cell, or (*3*) a naturally empty honeycomb cell (control). The amount of EBO was based on previous work (He et al., 2016; Maisonnasse et al., 2009; Traynor et

al., 2014) and a small pilot study where we supplemented larval cells with EBO across a concentration gradient and compared nurse visitation (see Appendix 2). We selected the EBO dose that increased visitation relative to untreated larval cells in this pilot test. In early trials of our main experiment (4 total rounds out of 12), we included larvae treated with two forms of e-β-ocimene, a pure form (Toronto Research Chemicals, O150025) and a racemic mixture used in previous studies (Sigma, W353901; as used in He et al. 2016). Early results did not suggest a difference between the two types, so we treated results from both EBO sources the same in analysis and continued using only the racemic mixture in later trials (see Appendix 2).

Because larval age impacts nurse bee visitation behavior, we standardized larval age across the entire experiment. To do this, we chose a honey bee colony that was not otherwise used in the experiment (a different queen was used for each experimental round). We located the queen and placed her in a cage with an empty honeycomb frame (standard deep frame, approx. 19" x 9-1/8") for 24 hours to allow her to lay eggs. The cage has holes that are too small for the queen to pass through but large enough to allow workers access to the frame and larvae. Following the 24 h period, the queen was released back into the hive and the frame was placed back in the cage to prevent further laying (Rittschof et al., 2015). When the eggs had hatched and the larvae on the frame were approximately two days old (96-120 hours post-laying), we removed the frame from its natal colony and performed the larval treatments. We assigned up to thirty cells on the frame to one of three treatments (see above, N=approximately nine cells of each treatment).

The location of the cells for treatment on the honeycomb frame was necessarily constrained by the laying pattern of the queen. We selected cells covering the entire width

of the brood area since proximity predicts similar offspring age. We avoided selecting focal cells that were immediately adjacent when possible to minimize potential interference of the EBO between cells, since the EBO was pipetted on the wall of the cell. Cells that contained larvae were randomly assigned to the unaltered or EBO treatment. Control cells were selected as any naturally empty cells that were not adjacent to other treatment cells and were distributed across the brood area as evenly as possible based on the queen's laying pattern.

### 3.2.4 Nurse collection and observation hive setup

We inserted the honeycomb frame containing our treated larvae into the selected nurse source colony (either high-aggression or low-aggression, see above) for 10 minutes to draw nurse bees onto the frame (as in He et al. 2016). The frame was then removed and placed in the top portion of an Ulster observation hive (Fig. 3.1). This type of hive has an enclosed 5-frame, queen-right colony (known as a "nuc") in a wooden box below a single glass-paneled viewable frame mounted on top. The queenright colony provides the blend of typical hive and queen pheromones that are required for the nurse bees to behave normally, as bees quickly begin to change their behavior if they detect that their colony is queenless (Butler, 1954; Cejrowski et al., 2018). The top and bottom portions of the Ulster hive are separated by a mesh screen that allows air to pass freely and some physical contact between the bees (which is required for queen pheromone transfer; (Ferguson & Free, 1980) but does not allow the bees to mix. We did not observe any overt aggression between bees at the nexus of the top and bottom portions. The same small colony was maintained in the bottom portion of the hive throughout the season, one for each year. Throughout the

experiment, the colony was kept inside of a small shed but was allowed to forage freely through a tube that connected to the outside (including during assays). Nurses in the top portion of the hive were provided with supplemental honey via a drip feeder and bee-collected pollen rolled into balls (Betterbee) *ad libitum*. The honey feeder was removed during the acclimation period and the ~35 min behavioral assay and video recording (see below), though the nurses would still have access to any food that had previously been stored on the frame and were able to exchange food with the lower hive bees via trophallaxis.

Figure 3.1

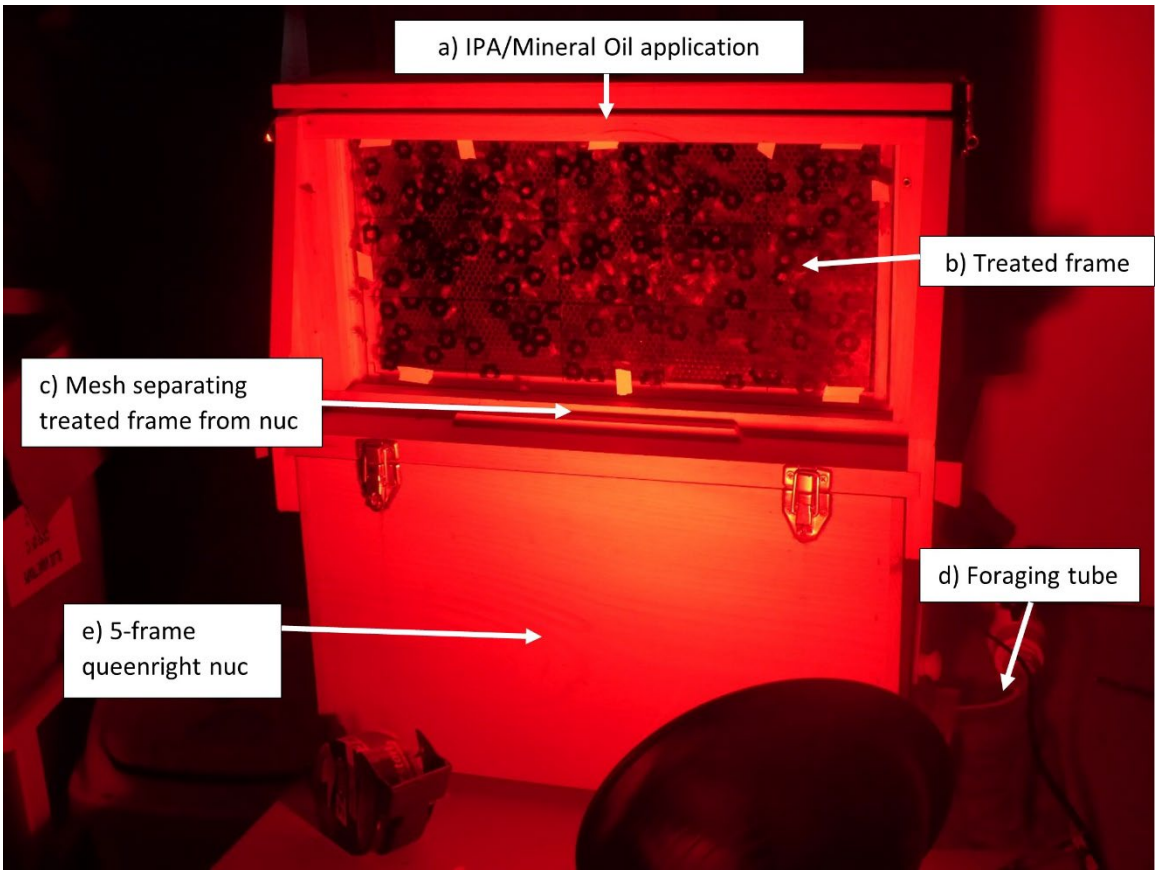


Figure 3.1. Picture of observation hive set-up under red light. a) Alarm pheromone (isopentyl acetate, IPA) or mineral oil as a control was applied to a small piece of filter paper that was placed on a mesh ventilation hole at the position indicated by the arrow; b) The treated frame with the nurses from high- and low-aggression colonies can be seen. A transparent sheet with guidemarks covers the outside of the observation hive to highlight the selected empty, larval, and begging-signal-augmented larval cells; c) The position of the mesh section that separates the treated frame from the nuc (a small, five-frame, queenright colony). The mesh allows smell signals and limited physical contact between the target nurses and the bees from the nuc but does not allow them to mix; d) Plastic and PVC tube that terminates outside of the shed and allows the bees in the nuc to forage freely. This tube was always open, even during assays, but the nurses on the top frame could not leave due to the mesh that separated them from the nuc; e) The nuc that provided the normal suite of background social signals found in queenright colony that is necessary for typical nurse behavior to occur.

Once the larval frame with nurses was placed in the top of the observation hive, the hive was kept under red light and allowed to acclimate for at least thirty minutes and until we observed normal nursing behavior. We placed a transparent sheet of plastic with guide marks over the side of the observation window to allow video scorers (see below) to identify the treated cells.

### 3.2.5 Nurse behavior assay and recordings

We recorded nurse bee behavior using a Panasonic HC-V770 video camera. In total, we video recorded the larval frame and nurse bee behavior continuously for about 35 min following the acclimation period. This included 2-5 minutes before an application of IPA or mineral oil control, and 30 min following application. The initial 2-5 min period was included to allow ample time to set up the camera properly and prepare and apply the IPA/mineral oil application. We analyzed the first 10 minutes following IPA or mineral oil application to assess how nurse behavior changes as a function of source colony aggression, cell EBO treatment, and IPA exposure (see below).

The alarm pheromone exposure treatment involved pipetting 10uL of mineral oil (control, Sigma, M8410) or a 1:100 dilution of isopentyl acetate (IPA, Sigma, 112674) in mineral oil onto a small piece of filter paper which was then immediately placed over a screened ventilation hole in the center of the top of the larval frame with nurses (see Fig 3.1) (Collins & Rothenbuhler, 1978). This amount of IPA falls within the range of a realistic dose of alarm pheromone that would be released by bees responding to an aggressive threat based on the sting-equivalent dose per bee, though we used a lower dose than in the colony-level aggression assays due to the proximity of the exposure point to the nurses (Allan et al., 1987). The location of nurse bees and the brood nest inside a honey bee colony can vary from a few centimeters from the entrance to more than half a meter away, but on average nurses are more distant from alarm cues than guards or soldiers. During each experimental round, we treated nurses once with IPA and once with mineral oil. The IPA and mineral oil applications were applied to the same group of nurses in a random order spaced 24h apart to allow recovery from the stimulus (Alaux et al., 2009; Collins & Rothenbuhler, 1978).

### 3.2.6 Ethical note

All honey bee colonies used to source bees for this study were maintained according to recommendations set forth by the Honey Bee Health Coalition. These recommendations are designed to minimize ecological impacts of beekeeping, as well as colony mortality and stress resulting from inadequate nutrition and/or high parasite and disease pressure. No permits, licenses, or pre-approval at the level of the institution or government were required to carry out this study. Nonetheless, we minimized our stress and mortality impacts by

using no more colonies than necessary to obtain a reasonable number of observations with a robust experimental design; the processes used to move and house the bees in this experiment are all standard practices. Worker bees were gathered with minimal disturbance based on which ones chose to enter the experimental frame. Frames with adult bees were moved from the source hive to the observation hive inside a dark, insulated box to minimize distress. The observation hive environment is very similar to a normal hive in terms of conditions such as temperature, humidity, density of individuals, and sensory enrichment, thus it is a low-stress experimental context. Furthermore, all manipulations surrounding the observation hive (introduction to the hive, maintenance, experimental procedures, and removal at the end of the study) were carried out under red light conditions to further prevent any additional stress (also a common practice). Throughout the experiment, bees had access to natural food sources *ad libitum* to minimize the possibility of nutritional stress. Exposure to alarm pheromone induces aggression, which could be considered a stressed state, but exposure to alarm pheromone is common in natural conditions at the level used in our study. All bees were returned to their natal hives upon completion of the observations. Few individuals died during the study, and the removal and return of experimental bees had no discernable impacts on their home colonies.

### 3.2.7 Behavior scoring from videos

The videos were scored by observers who were blinded to the nurse source colony, the identity of the treatment cells, and the IPA versus mineral oil application. To score the data on nursing behavior, the observers began watching from the moment the IPA or mineral oil was applied (excluding the first 2-5 minutes of preparation in the video) and

observed for 10 minutes. The observers scanned each treatment cell (N=~30, see above) for nursing behavior, where a worker bee placed her whole head (and in some cases, thorax or abdomen) inside one of the treatment cells. Observers did not track individual nurses, but rather tallied the total amount of nursing attention a cell received. Observers recorded the number of times any nurse bee placed her head in each cell type, as well as the timestamp and duration of each visit to the nearest second. Inspection of the video scoring revealed that false positives were far more common than false negatives due to a grooming behavior that can look similar to a nursing visit. In the grooming behavior, the nurse necessarily angles her head downward (appearing to go towards a cell) as she lifts her abdomen high, rubbing her back legs together and against the bottom and sides of her abdomen. In a nursing visit, the bee only moves her head downward while keeping her abdomen mostly parallel to the comb's surface and without rubbing her legs (unless she climbs fully into the cell, which is unmistakably nursing behavior). A separate observer who was trained to distinguish the grooming behavior checked each recorded nurse visit to determine if it was a true observation.

We additionally analyzed the activity level of the bees surrounding the presentation of alarm pheromone (or its mineral oil control counterpart). For this analysis, observers would count the number of times any bee crossed a horizontal line on the frame and would note the direction of the cross (up or down). These observations were completed for 15 seconds at six time points for each video: 2 minutes before stimulus presentation, 1 minute before stimulus presentation, directly at the moment of stimulus presentation, 1 minute after stimulus presentation, 2 minutes after stimulus presentation, and 5 minutes after stimulus presentation.

### 3.2.8 Statistical analysis

We performed statistical analyses with R version 4.1.2 (R Core Team, 2021). To evaluate how pheromone treatments and source-hive aggression impacted the frequency of nurse visits and the latency to the first visit, we used the "glmmTMB" package to create generalized linear mixed models (GLMMs) with a negative binomial distribution with quadratic parameterization (Brooks et al., 2017; Hardin et al., 2007). Our response variable for this analysis included nursing observations following the IPA or mineral oil application. We included nurse source colony aggression level (high vs low), alarm pheromone application (IPA versus mineral oil), cell treatment (larva, larva with EBO, empty), and their interactions as fixed effects. All interactions (including the three-way interaction) were included in the global model. We additionally included source colony ID (a unique identifier of the colony the nurses came from), year, and IPA vs mineral oil application order as random effects.

In our experiment, source colonies were derived from a variety of genetic strains (see "*Honey bee sources"* above). Because genetic strain is correlated with aggression in some studies (Alaux et al., 2009; Harpur et al., 2020; Locke, 2016), we considered including it as a factor in our models. However, preliminary examination of the data showed that the high- and low-aggression source colonies used in our experiment were distributed evenly across strains, suggesting no clear association between aggression and strain. Therefore, we omitted genetic strain from our models.

We took the global model and created alternative candidate models by progressively removing interaction terms. We then used AICc-based model selection

criteria to select the final model with the "AICcmodavg" package (Mazerolle, 2020). Model diagnostics were assessed using the "DHARMa" package, which includes a QQ plot, KS test, dispersion test, outlier test, within-group uniformity test, and Levene test (Hartig, 2022). We used the "car" package to run a Type III ANOVA on the final model to estimate significance values (Fox & Weisberg, 2019). We used the "performance" package to examine whether our dataset showed zero-inflation (Lüdecke et al., 2021). Post-hoc comparisons were carried out using the "multcomp" and "emmeans" packages for Tukey tests and estimated marginal means (EMM) comparisons respectively (Hothorn et al., 2008; Lenth, 2022).

To evaluate the duration of nurse visits, we categorized visits into groups based on how long the visit lasted. When a nurse enters a honeycomb cell with a larva (called a "visit"), she may be quickly checking the feeding status or health of the larva, feeding the larva, or sleeping or performing thermoregulation activities (Gilliam et al., 1983; Lindauer & Watkin, 1953; Siefert et al., 2021). The nature of the nurse's visit can be assessed using the duration of the visit: anything shorter than 20 seconds is likely a brief check to assess the health of the larva and/or its feeding status (hereafter "inspection"), anything between 20 seconds to 3 minutes is characteristic of larval feeding (hereafter "feeding visit"), and anything longer than 3 minutes is an indication of sleeping or thermoregulation (hereafter "sleeping/thermoregulation") (Brouwers et al., 1987; Gilliam et al., 1983; Lindauer & Watkin, 1953; Siefert et al., 2021). Similar to previous work (Brouwers et al., 1987; Huang & Otis, 1991; Lindauer & Watkin, 1953; Riessberger & Crailsheim, 1997; Schmickl et al., 2003), we further subdivided the "inspection" category into two parts: "short inspections" (1 second or less, where a nurse is likely very briefly using olfactory cues to rule out if a

larva is hungry or diseased before moving on) and “long inspections” (2-20 seconds, where a nurse is likely taking more time to assess how much food a larva has to determine if it requires more). Within each group, we used individual chi-square tests to compare the levels for each of our major factors (nurse source hive aggression, IPA application, cell type, as described above). None of these initial comparisons were significant, so we chose not to further examine the duration data for interaction effects among factors. We performed our tests using chi-square tests of independence with the chisq.test() function (R Core Team, 2021).

To assess the activity level, we built linear mixed models using the lme4 package (Bates, 2015). The data were log-transformed to improve the data distribution. The number of crosses was our response variable. We used a similar AIC-based process to achieve our final model from a global model that contained aggression, alarm pheromone application, timepoint, direction and all possible interactions as fixed effects, plus colony ID and treatment order as random effects. Model diagnostics and significance values were determined as described above. We again used the emmeans() package to carry out post-hoc comparisons.

Figures were generated using the ggplot2 package (Whickam, 2016).

## 3.3 Results

### 3.3.1 Impacts of competing pheromone information on the number of nurse visits

We built a GLMM to examine how competing pheromone information impacts the number of nurse visitations to larval cells. The fixed effects that were included in our final model are shown in Table 3.1. The full global model and the random effects for the final

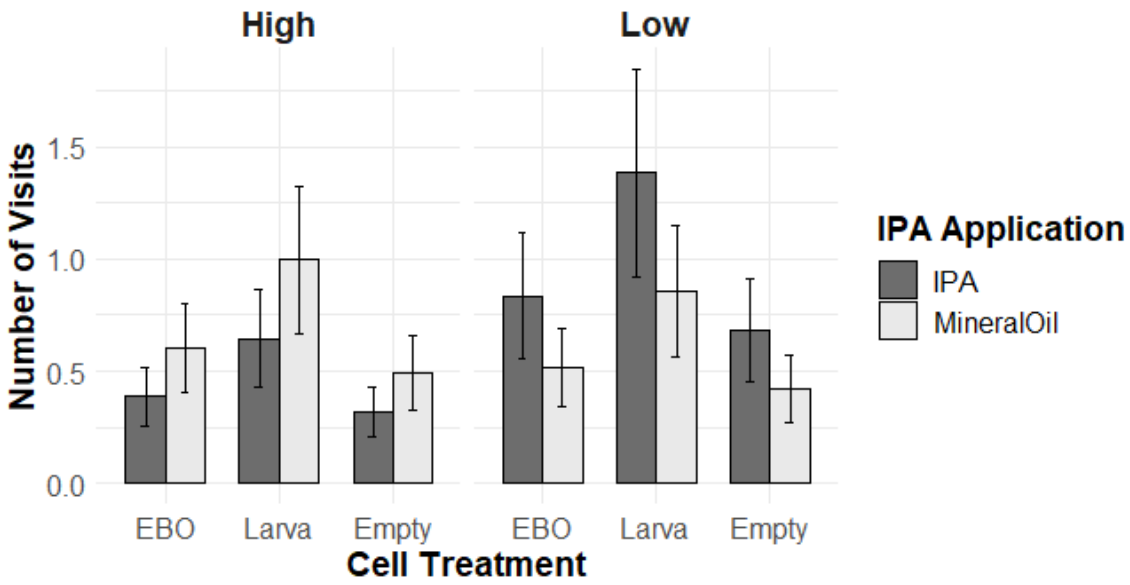
model can be found in Appendix 2. As expected, cell type (variation in begging pheromone emission: empty cell, larva, larva + EBO) significantly impacted the number of nurse visitations (ANOVA: Wald $X^2_2$=16.6, *P*= 0.0002; range=0-28 visits, Fig 3.2A). We expected that EBO-treated larval cells would show the greatest number of visits, followed by untreated larval cells and empty cells (He et al., 2016). However, while untreated larval cells showed significantly more visits than empty cells (Tukey test: *P*<0.001), EBO-treated larval cells received significantly *fewer* visits than untreated larval cells and were not significantly different from empty cells (Tukey test: Larva-EBO, *P*=0.01; EBO-Empty, *P*=0.53). We suspected that this phenomenon might have been caused by EBO-treated cells being visited first due to the heightened strength of the signal. Most cells that were visited at least once received only one visit (one visit: 54%; all other numbers of visits: 46%). If these early visits occurred during the acclimation period when we were not observing visits, the result would be reduced visits during the actual observation window. We therefore analyzed the proportion of cells that received zero visits during the observation window (versus cells that received any number of visits). Though the dataset overall was not zero-inflated (ratio of observed to predicted zeroes=1.01), we found that there were nearly 50% more zeroes in the EBO-treated larval cells than untreated larval cells (Fig. 3.3) (Chi-square test: $X^2_2$=19.37, *P*=0.00006; post-hoc comparisons: EBO vs. Larva, adjusted *P*-value=0.00005; Larva vs. Empty, adjusted *P*-value=0.01; EBO vs. Empty, adjusted *P*-value=0.15). Figure 3.2B shows the rate of visitation only to cells that received at least one visit.

Table 3.1 Fixed effects from the final GLMM used to evaluate how competing pheromone information and nurse source colony aggression impact the number of nurse visits with Wald $X^2$ values, degrees of freedom (DF), and ANOVA-determined *P*-values.

| Factor | Wald $X^2$ value (DF) | *P*-value |
|---|---|---|
| Nurse source colony aggression | 0.98 (1) | *P*=0.32 |
| IPA application | 0.07 (1) | *P*=0.79 |
| Cell type | 16.6 (2) | ***P*=0.0002*** |
| Nurse source colony aggression*IPA application | 8.2 (1) | ***P*=0.004**** |

Figure 3.2

A


High
Low
Number of Visits
1.5
1.0
0.5
0.0
EBO
Larva
Empty
EBO
Larva
Empty
Cell Treatment
IPA Application
IPA
MineralOil

B

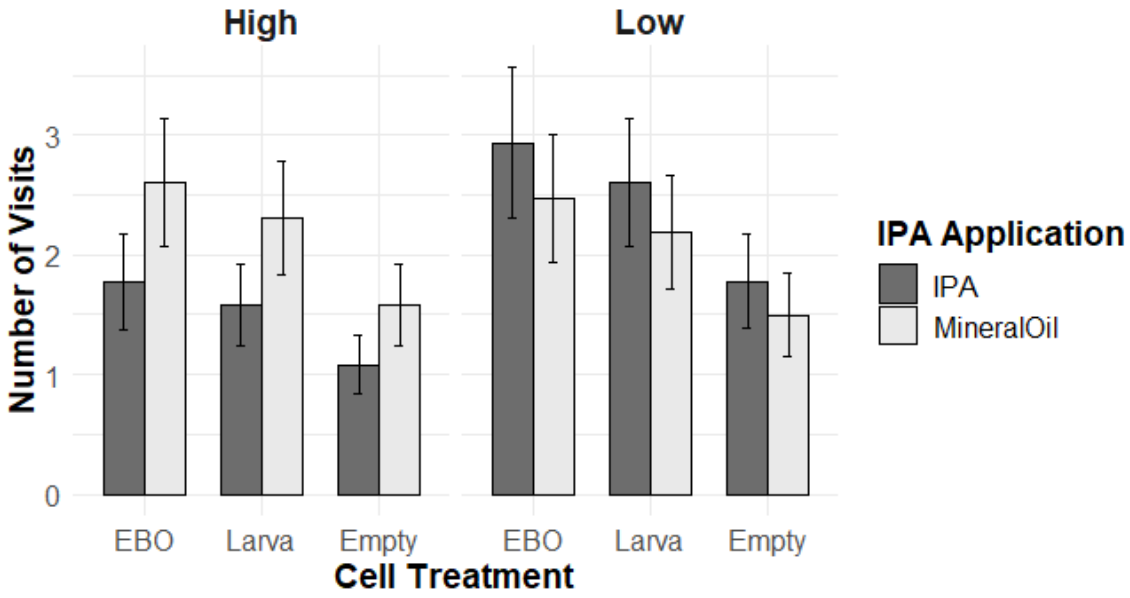

High
Low
Number of Visits
3
2
1
0
EBO
Larva
Empty
EBO
Larva
Empty
Cell Treatment
IPA Application
IPA
MineralOil

Figure 3.2. Nurse bees from high-aggression colonies decreased their visits in the presence of alarm pheromone, while nurses from low-aggression colonies did not. A) Least-squares mean ± SE visits by nurses from high- and low-aggression colonies to cells that contained a larva with added e-β-ocimene (EBO), an untreated larva, or an empty cell on days where alarm pheromone (isopentyl acetate, IPA) or mineral oil was applied. The statistical model showed a significant main effect of cell treatment and a nurse source colony aggression*IPA application interaction effect. B) The original finding, that nurse bees from high-aggression colonies decreased their visits in the presence of alarm pheromone while low-aggression nurses did not, remains true when removing cases where a cell received zero visits.

Figure 3.3

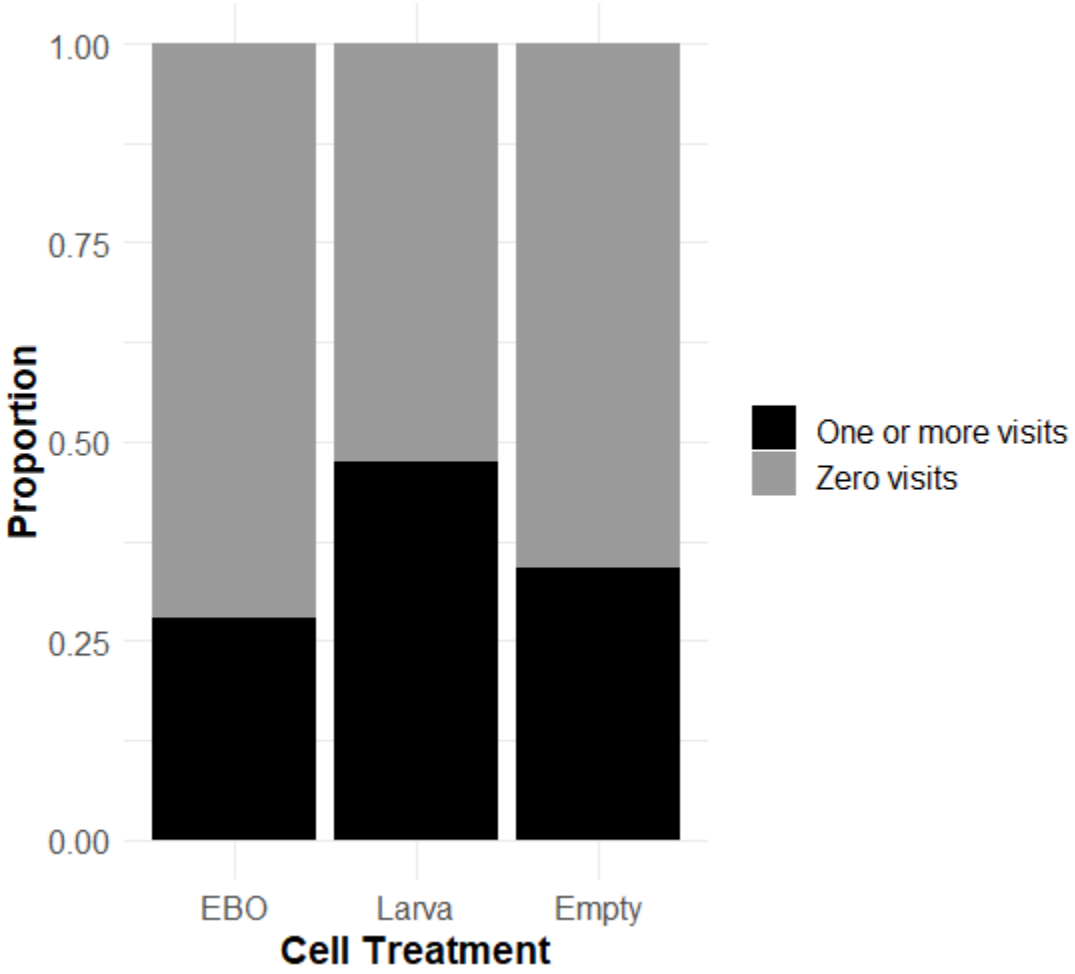


Figure 3.3. A higher proportion of larval cells treated with e-β-ocimene (EBO) received zero visits than untreated larval cells. Proportion of each cell treatment (a larva with added e-β-ocimene [EBO], an untreated larva, or an empty cell) that received zero visits versus cells that received any number of visits.

In addition to their response to brood signals, we found evidence that nurses also react to alarm pheromone, and that their colony of origin influences this behavior: we found a significant interaction between nurse bee source colony aggression and IPA application on the number of nurse visits (ANOVA: Wald $X^2_1$=8.2, *P*= 0.004). Nurse bees from high-aggression colonies decreased the number of visits to larvae in the presence of IPA relative to the mineral oil control, while nurses from low-aggression colonies did not show this change (Fig. 3.2A). Nurses from high aggression colonies made 35% fewer visits in the presence of IPA compared to the mineral oil control (High aggression: EMM log contrast= -0.44, *P*=0.045). In contrast, nurses from low aggression colonies showed a non-significant trend towards increasing their visits in the presence of IPA, making 62% more visits during trials with IPA than trials with mineral oil (Fig. 3.2A) (Low aggression: EMM log contrast= 0.48, *P*=0.055). We found no evidence that the response to IPA was influenced by begging cues. Rather, IPA decreased nurse activity with no additional influence of EBO treatment. This finding, that nurse bees from high-aggression colonies decreased visits in the presence of IPA while nurses from low-aggression colonies did not, remained true when considering only cells that received one or more visits (Fig. 3.2B).

### 3.3.2 Impacts of competing pheromone information on the duration and timing of nurse visits

Almost all of the visits we observed (505/519, 97%) were inspections. Of these, 341 (67.5%) were short inspections (<1 second long) and 164 (32.5%) were long inspections (2-20 seconds long). The ratio of short to long inspections was not affected by any of our explanatory variables (Chi-square test: nurse source colony aggression: $X^2_1$=0.06, $P$=0.81; IPA application: $X^2_1$=0.03, $P$=0.87; cell type: $X^2_2$=2.33, $P$=0.31).

Because we found an interaction effect between nurse source colony aggression and IPA application in the analysis of the number of nurse visits (above), we additionally performed a chi-square test on nurse source colony aggression separated out by IPA application (one for only IPA trials, one for only trials with the mineral oil control). The number of visits was similar within the mineral oil application comparing between high- and low-aggression nurse source colonies (Fig. 3.2). Meanwhile, there was a strong difference between the number of visits by nurses from high- and low-aggression colonies within the IPA application (Fig. 3.2). Dividing the visit duration chi-square tests in this way would allow us to see if there were a similar pattern in the duration data. However, these tests were additionally nonsignificant (Chi-square test: nurse source colony aggression, IPA only: $X^2_1$=0.41, $P$=0.52; nurse source colony aggression, mineral oil only: $X^2_1$=0.03, $P$=0.85).

We identified 12 feeding visits (2.3% of total visits), a frequency that is consistent with previous observations of nursing behavior (Brouwers et al., 1987; Huang & Otis, 1991; Lindauer & Watkin, 1953). Nurses from low aggression source colonies performed feeding visits at over three times the frequency of nurses from high aggression source colonies, but likely due to the small total number of feeding visits, this pattern was not

statistically significant (Chi-square test: $X^2_1$=2.88, *P*=0.09). Neither IPA application nor cell type showed significant differences in the number of feeding visits (Chi-square test: IPA application: $X^2_1$=0.43, *P*=0.51, cell type: $X^2_2$=0.06, *P*=0.97). Only 2 out of 519 total visits (0.4%) fell into the sleeping/thermoregulation category, precluding further statistical analysis.

We also examined the timing of visitations. We first tested whether nurse source colony aggression, IPA application, and cell type impacted the latency to the first nursing visit for each cell that received at least one visit. The latency to the first visit was not significantly affected by any of these explanatory variables nor their interactions (see Appendix 2, Fig A2S4). Additionally, we visually examined the distribution of all visits within the 10-minute window. We saw no clear directional trend, suggesting that the depression of visitation seen in high-aggression colonies on IPA days lasted at least 10 minutes (Fig. 3.4). Finally, we assessed whether visitation was different within each combination of aggression level and IPA application based on treatment order (i.e. if IPA was applied on the first or second day). We found no effect of treatment order within any combination of IPA application and source colony aggression (High aggression IPA: EMM log contrast= 0.02, *P*=0.96; high aggression mineral oil: EMM log contrast= -0.54, *P*=0.22; low aggression IPA: EMM log contrast= 0.29, *P*=0.62; low aggression mineral oil: EMM log contrast= -1.14, *P*=0.08). Because we saw no difference in the rate of visitation on mineral oil control days when they fell after the alarm pheromone treatment (as opposed to before), we can infer that the rate of nursing behavior had returned to baseline within 24 hours of a perceived threat.

Figure 3.4

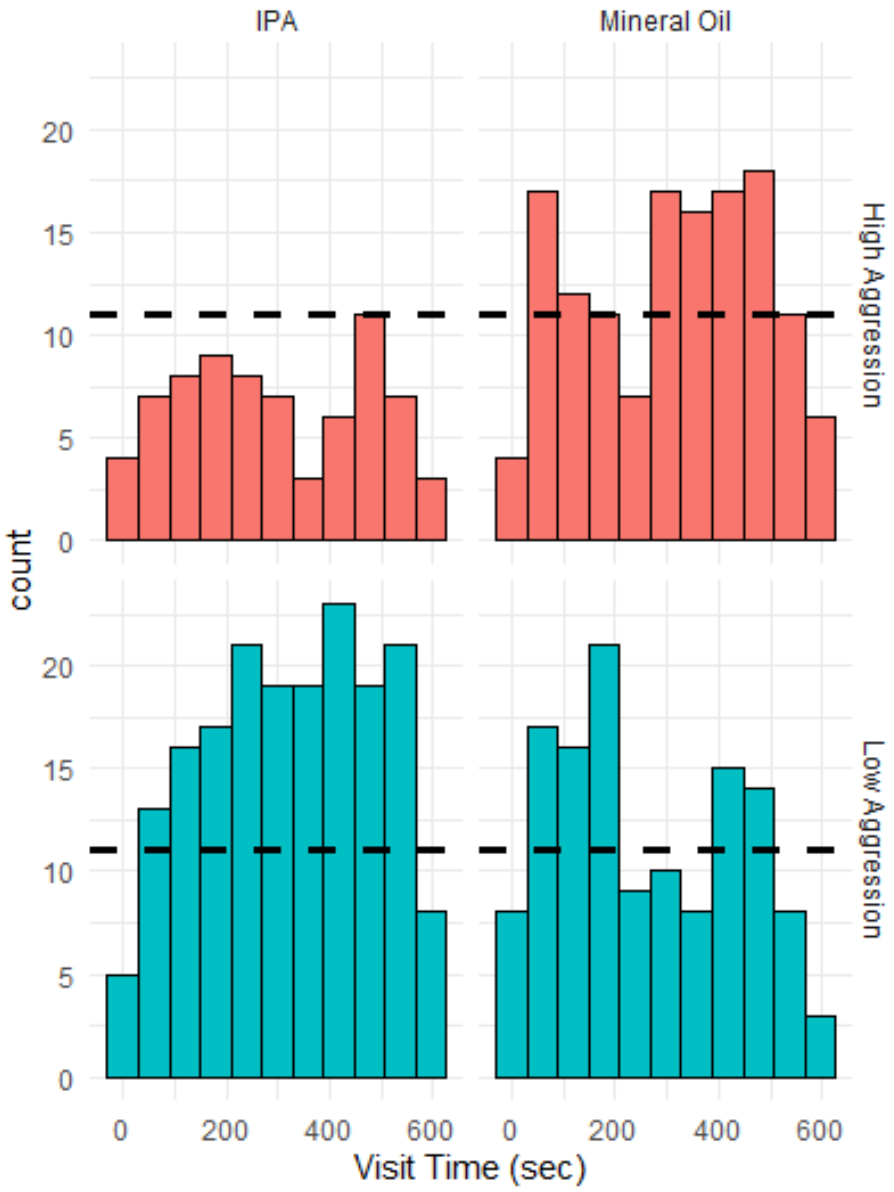



Figure 3.4. The rate of nursing visits was relatively even over the 10-minute observation window, with the rate of visitation in high-aggression colonies on alarm pheromone (IPA) days remaining depressed below the rate on mineral oil days for the duration. Histogram showing the time in seconds of visits to all cell types. High aggression above in red, low aggression below in blue, IPA on the left and mineral oil on the right. Dashed line represents the mean value per bin on mineral oil (control) days.

### 3.3.3 Impacts of alarm pheromone on the activity level of honey bees

To gain a clearer understanding of the behavior of the bees in the immediate aftermath of the alarm pheromone presentation, we measured the activity level and gross movement patterns (towards versus away from the site of the alarm pheromone) before and after stimulus presentation. We found that the application of alarm pheromone affected the total activity level of bees in low-aggression colonies, but not high-aggression colonies. Our final linear mixed model included aggression, alarm pheromone application, direction, and the interaction between aggression and alarm pheromone application as fixed effects, with colony ID and treatment order as random effects. We found a significant interaction effect between aggression and alarm pheromone application, suggesting that the addition of alarm pheromone affects the overall activity level of bees from different colony aggression levels in different ways (ANOVA: Nurse source colony aggression: Wald $X^2_1$=4.2, $P$= 0.04; alarm pheromone application: Wald $X^2_1$=0.10, $P$= 0.76; direction: Wald $X^2_1$=9.0, $P$= 0.002; nurse source colony aggression*alarm pheromone application interaction effect: Wald $X^2_1$=60.7, $P$< 0.0001). An estimated marginal means post-hoc comparison revealed that overall activity level in low-aggression colonies was more than halved on alarm pheromone days compared to mineral oil days (8.0 crosses per 15 seconds compared to 16.4 crosses per 15 seconds). The activity level in high-aggression colonies was unaffected overall (12.4 crosses per 15 seconds versus 12.2 crosses per 15 seconds; high aggression: EMM log contrast= 0.02, $P$=0.76; low aggression: EMM log contrast= -0.72, $P$<0.0001). We additionally tested the pairwise differences between directions at each timepoint (i.e. up versus down at each timepoint for each combination of aggression and alarm pheromone application). We found only one timepoint with a difference: in high-

aggression colonies on days where alarm pheromone was applied, during the 15 seconds immediately following the application of the pheromone, bees were significantly more likely to cross up (towards the pheromone source) rather than down (away from the pheromone source). They crossed up twice as often as down during this timepoint (EMM log contrast= 0.68, *P*=0.0096 (corrected with the Bonferroni method for the large number of comparisons); Fig. 3.5; see Appendix 2, Table A2S2 for a table of all *P*-values). The bees had returned to equal numbers of crosses in each direction by the next measured timepoint one minute later.

Figure 3.5

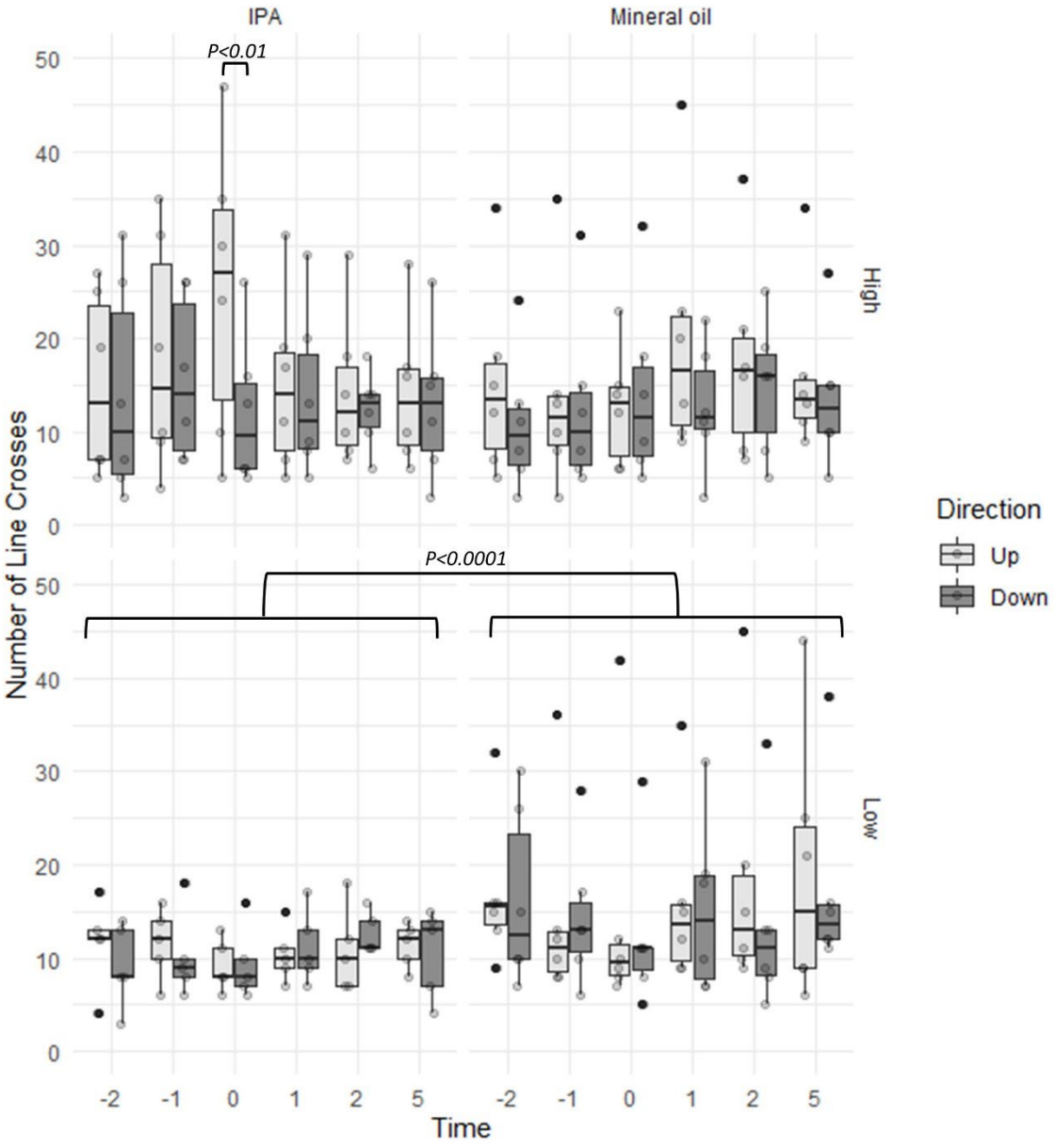

IPA
Mineral oil
P<0.01
P<0.0001
High
Low
Number of Line Crosses
Time
Direction
Up
Down
0
10
20
30
40
50
-2
-1
0
1
2
5

Figure 3.5: Total activity was suppressed in low-aggression colonies on days when alarm pheromone (IPA) was applied. In addition, bees in high-aggression colonies more frequently moved towards the alarm pheromone stimulus ("Up") than away from it ("Down") during the 15 seconds immediately following alarm pheromone application. Boxplots of the number of times any bee crossed a horizontal line in the viewable area over a period of 15 seconds. This activity measurement was taken six times (noted as "Time" on the x-axis), with "0" being the moment of stimulus presentation of either IPA or mineral oil. Other measurements were taken at two and one minutes before the stimulus as well as one, two, and five minutes after the stimulus presentation. High-aggression colonies are displayed above with low-aggression colonies below. Alarm pheromone application is displayed on the left ("IPA") and the mineral oil control is displayed on the right. The number of line-crosses up (towards the stimulus) is displayed in light gray and the number of line-crosses down (away from the stimulus) is displayed in dark gray.

## 3.4 Discussion

Here we show in a naturalistic colony context that nurse bees respond to social cues related to a separate specialization, colony defense. Even though nurses have a higher threshold for alarm response compared to defensive specialists (Robinson, 1987a), alarm information caused significant variation in the frequency of visits to larvae regardless of the intensity of begging cue emission. Importantly, we found that another source of variation in response thresholds to alarm signals, colony-of-origin, influenced this outcome: nurses collected from high aggression colonies changed their larval care behaviour in the presence of alarm pheromone, while nurses from low aggression colonies did not show this pattern. Overall, these results suggest behavioral specialists attend to a wider range of social cues than previously appreciated, and that social and ecological information (e.g., predator threat levels) may have far-reaching and multigenerational colony-level impacts.

There are at least two mechanistic explanations for why the nurses from high-aggression colonies change their larval care behaviour in the presence of alarm pheromone. First, it is possible that alarm pheromone interferes with their ability to detect cues emitted

by larvae, decreasing their nursing response (i.e. masking, olfactory receptor antagonism) (Oka et al., 2004; Rosa & Koper, 2018). Future studies employing electroantennography could explicitly test if there is any role of antennal sensitivity in the simultaneous detection of larval cues (such as e-β-ocimene) and alarm pheromone as well as the role that colony-level aggression plays in modulating this sensitivity. A second explanation is that nurses are able to detect larval cues irrespective of alarm pheromone presence, but are preferentially responding to the alarm pheromone cue (i.e. distraction) (Rosa & Koper, 2018). This hypothesis is partially supported by our finding that bees from high-aggression colonies more frequently moved towards the source of the alarm pheromone in the time period immediately after it was applied. Such an outcome may be particularly relevant in the context of nest defense, which relies on the successful recruitment of a critical mass of workers to fend off an attack (Breed et al., 2004). For example, several studies show that one to two day old workers (which are relatively insensitive to alarm pheromone; (Robinson, 1987a) respond behaviorally to alarm pheromone and intruder attack (Collins, 1980; Rittschof, 2017). Future studies could investigate the neural basis of prioritization of the alarm response in nurses and other workers. It would also be interesting to assess whether the response to non-specialist cues demonstrated here is limited to alarm pheromone (due to its critical role in nest defense) or occurs more broadly.

Our finding that the nurse response to alarm cues depends on the colony-of-origin's aggression level suggests behavioral expression is the result of complex interactions between individual sensitivity thresholds and proximal social cues. Sensitivity thresholds can be shaped by genetic variation as well as social and ecological information (Calderone & Page, 1988; Page & Robinson, 1991; Scheiner & Erber, 2009; Wilson, 1985). Animals

from fish to birds to mammals tailor both their signal production and cue response to health and body condition (Bachman, 1993; Brown et al., 2004; Burkhard et al., 2018; Seltmann et al., 2012). Both genetic and environmental mechanisms appear to influence alarm cue response in honey bees (Alaux & Robinson, 2007; Alaux et al., 2009; Guzman-Novoa & Page, 1994; Hunt et al., 2003) For example, Rittschof et al. (2015 and 2019) showed that the developmental colony environment has lasting impacts on the threshold of individual responsiveness to alarm pheromone as well as immune system activity and pesticide tolerance. Alternatively, Giray et al. (2000) showed that genetic variation in worker developmental rate can cause differences in colony level aggression and foraging behaviour. Thus, the nurses in our study may differ in their aggression response thresholds due to genetic differences or environmental factors such as infection, stress exposure, or ecological conditions (Carr et al., 2020; Couvillon et al., 2008; Downs & Ratnieks, 2000; Garbuzov et al., 2020). High-aggression colonies may overall prioritize nest defense, manifesting as both strong guard/soldier response to threats and nurse prioritization of alarm cues over nursing cues. Interestingly, these results combined with the results of Rittschof et al. 2015 raise the possibility that threshold differences in alarm response among nurses could in turn influence the behavior and health of the subsequent worker generation.

Though it was not statistically significant, we note that the nurses from low aggression colonies showed an increase in visitation in the presence of alarm pheromone. This effect is particularly remarkable given that total activity was suppressed in low-aggression colonies on days where alarm pheromone was applied relative to the control; that is, there were more nurse visits to cells at the same time as less total movement. If the

trend of increased visitation is a true phenomenon rather than noise in the data, one possibility is that it is due to olfactory priming. Olfactory cues can in rare cases be enhanced by particular background odorants (Deisig et al., 2014; Schröder & Hilker, 2008). For example, male *Helicoverpa zea* moths show increased activity of a neuron specifically tuned to the female sex pheromone in the presence of linalool or hexanol, despite these two chemicals not activating that neuron in the absence of the pheromone (Ochieng et al., 2002). If these dynamics exist in this system, the presence of alarm pheromone could have enhanced the response to other larval cues. Additionally, a recent study identified that young larvae (instars 1-4) emit small amounts of isopentyl acetate (IPA, an important constituent of the honey bee alarm pheromone and the chemical used in our study) (Noël et al., 2023). It is therefore possible that this chemical serves a second function as a larval signal in addition to an aggressive signal, although this idea has not been explicitly tested. The addition of extra IPA in our study could have caused nurses in low-aggression colonies to overestimate the number of larvae present, generally increasing their nursing effort and leading to a higher number of visits. What is not clear from this scenario is how the directional dynamics of the pheromone application would have affected this process (as the alarm pheromone was applied at the top of the frame rather than directly over the larval area) as well as why this effect would have been limited to nurses from low-aggression colonies.

In the current study, we showed variation in nursing behavior in response to a single, uniform application of alarm pheromone. However, the consequences of this variation may depend on the patterns of alarm cue signaling and response over the course of larval development, which lasts 5-6 days. For example, high- and low-aggression

colonies could differ in the amount and timing of alarm pheromone release in a natural hive setting. High aggression colonies may be exposed to more threats or, being more sensitive to threats, may release alarm pheromone more frequently (Alaux et al., 2009; Guzmán-Novoa et al., 2004). Additionally, additive effects of alarm pheromone release, where each responder releases additional alarm pheromone, can explain colony-level variation in defensive aggression (Guzman-Novoa & Page, 1994). Such effects could increase the total amount of nurse bee alarm pheromone exposure in high-aggression colonies, even during a single antagonistic encounter.

Given the pheromone signaling dynamics in high-aggression colonies, the total amount of disrupted nursing time could add up substantially over the 6-day course of larval development. However, nurse bee response to alarm pheromone may also be dynamic over time. Animals that are repeatedly exposed to the same stimulus often show habituation, a decrease in the magnitude of the behavioral response across repeated exposures (Thompson & Spencer, 1966). Alternatively, sensitization can occur, where the magnitude of the behavioral response increases across repeated exposures (Minoli et al., 2012; Russo & Ison, 1979; Walters et al., 2001). Both habituation and sensitization can be affected by the frequency and the intensity of the stimulus given (Groves et al., 1969; Pilz & Schnitzler, 1996; Thompson & Spencer, 1966). Nurses—which specialize on brood care for several days before switching to other tasks—may be subject to either process, but these possibilities remain to be tested (Seeley, 1982). Understanding alarm cue release and response dynamics over extended timeframes will be required to interpret the extent to which nurse alarm pheromone response contributes to variation in individual larval development and colony-level phenotypes.

We did not examine whether the short-term variation in nurse visits in our study affected larval outcomes. An individual honey bee larva is fed a little more than once per hour on average (Brouwers et al., 1987; Huang & Otis, 1991). We measured the disruption by alarm pheromone for 10 minutes. Although the larval signaling response to starvation occurs rapidly (the begging signal is released within 30 minutes of food deprivation), mortality impacts require longer periods of deprivation, on the time course of hours (He et al., 2016). It is possible that a brief disruption in the rate of nursing could be made up for by a temporary increase in the nursing workforce or increased effort by individual nurses once the perceived threat has passed, resulting in similar amounts of total food provisioned across high and low aggression colonies (Charbonneau & Dornhaus, 2015; Charbonneau et al., 2017; Harbo, 1986). Our study design cannot fully address this possibility. The disruption in high-aggression colonies caused by the addition of alarm pheromone lasted the full ten minutes of observation, as the rate of nursing was depressed below the values seen on mineral oil days for the duration of our observation window. We also did not see an effect of treatment order—the rate of visitation on mineral oil control days was similar whether they fell before or after the alarm pheromone treatment day. We therefore can say that the rate of nursing had recovered to baseline within 24 hours after an alarm pheromone exposure. Thus, any increase in effort by the nurse bees would have had to have occurred between 10 minutes and 24 hours post-threat, if at all. Periods of food deprivation could also accumulate over time in high-aggression colonies. For example, colonies face limitations to their provisioning abilities in other contexts such as a shortage of workers dedicated to brood care (Eischen et al., 1982, 1983) or brief disruptions in pollen availability; these both specifically impact nurse visitation to the young larvae we

examined here (Schmickl & Crailsheim, 2002). These limitations can result in physiological effects like decreased progeny lifespan and protein content (reviewed in (Brodschneider & Crailsheim, 2010). Similar outcomes could occur in high-aggression colonies where nursing is disrupted by alarm pheromone.

The current study was premised on the idea that variation in nurse behavior and accompanying impacts on larval physiology may underlie an adaptive larval response to colony social or ecological conditions. In honey bees, there is evidence of subtle responses to larval food deprivation that may have adaptive value. For instance, adult workers deprived of food as older larvae show increased starvation resilience, juvenile hormone titers, and glycogen stores as adults (Wang, Kaftanoglu, et al., 2016). While this could reflect an adaptive physiological response to food scarcity, these characteristics also are associated with increased aggression in other honey bee studies (Pearce et al., 2001; Robinson, 1987a, 1987b). The adaptive value of such a shift in aggression is unknown, but it could give a competitive advantage to colonies under conditions of floral resource scarcity, as these circumstances increase the frequency of aggressive interactions among honey bee colonies (Garbuzov et al., 2020). Thus, nurses from high-aggression colonies may periodically and temporarily deprive larvae of food, causing increased aggression in response to environmental conditions (Rittschof et al. 2015). It is interesting to note that a variety of animals show a relationship between early-life nutritional deficits and adult aggression, suggesting a more general mechanistic and adaptive tie between these two characteristics (D'Eath & Lawrence, 2004; Randt et al., 1975; Shen et al., 2021).

Rather than the absolute degree of food deprivation, larval honey bees may also use the consistency of feedings in early-life as a source of information about the social or

ecological environment they will experience in the future. Feeding disruptions may introduce uncertainty into a larva's assessment about the status of the environment, shaping their adaptive developmental trajectory (Trimmer et al., 2011). For example, spatiotemporal heterogeneity in environmental resource conditions, perceived through unpredictable nursing, could lead to a pessimistic cognitive bias, i.e., an expectation of poor resource conditions. Because such conditions are typically correlated with increased competition among colonies (Downs & Ratnieks, 2000; Willingham et al., 2000), an adaptive larval response would include increased aggression (Fawcett et al., 2014). Modeling studies indeed suggest that temporal variation in the environment across generations can select for pessimism (McNamara et al., 2011). Similarly, empirical studies demonstrate a positive correlation between uncertainty and aggression along with other co-varying traits (see (Lewis, 2022; Mathot et al., 2012; Sih et al., 2015; Silk et al., 2019; Stamps & Frankenhuis, 2016); but see also (Benus et al., 1991). Future studies could investigate the mechanistic and adaptive consequences of variation in nurse visitation at both the individual and colony levels. For example, social insect colonies make decisions collectively, and it is possible that information acquired by other colony members may counteract or amplify the effects of nursing uncertainty experienced by cohorts of developing larvae (Marshall et al., 2009).

In addition to their response to alarm cues, our study suggests other sources of unexplored complexity in the cues nurses use to guide their interactions with larvae. For example, we observed that larval cells augmented with e-β-ocimene (EBO) were visited less frequently than unaltered larval cells. This effect could suggest signals other than EBO play a role in modulating nurse visits. These signals could include other olfactory signals

from the brood or the brood food as well as cues such as vibrations created as the larva moves and feeds (Heimken et al., 2009; Huang & Otis, 1991). Alternatively, there may be technical explanations for this effect. We found that a large proportion of cells in the EBO group received zero visits, possibly because visitations occurred outside our observation time window. Most cells that were visited during our observations received only one visit. EBO cells, which have the strongest begging signal, may have been visited by nurses preferentially when they were first introduced to the frame during the acclimation period and outside our observation timeframe. Although our EBO treatment mimicked previous studies, it is also possible that the presence of EBO repelled nurses from visiting cells. Follow-up studies should carefully examine how naturally emitted and supplemented EBO, in addition to other cues, alter nurse activity. For example, a two-choice experiment could directly compare nurse visitation preference for larvae with and without extra EBO.

The "cocktail party problem" describes the increased difficulty in attending to a particular stimulus in the face of competing information, particularly when that competing information is also socially relevant (such as how it is more difficult to filter out other human conversations than ambient noise (Cherry, 1953). Embedded within this framework, though, is recognition that highly relevant stimuli are able to break through the attention barrier, such as when a person's attention is diverted from a conversation by hearing their own name (Moray, 1959). Our research highlights that this phenomenon is true even in social insects. We did not find an interaction effect between adding additional begging pheromone and alarm pheromone treatment, but the larvae were still presumably releasing their own larval signals that the nurses were cueing into. Some nurse bees that were attending to this social information given off by the larvae were distracted by a

different social cue, alarm pheromone, while others continued to focus on larval cues in the face of this alternative social information. Furthermore, the bees from low-aggression colonies were evidently affected by the alarm pheromone (as shown by the drastic decrease in total activity), and yet they maintained the level of nursing effort while under this effect. Individual variation has been found in human studies of the cocktail party phenomenon and is related to factors such as working memory (Conway et al., 2001). The variation seen in our study was associated with the larger-scale social factor of colony-level aggression. It would be interesting to see if broader social context affects this phenomenon in humans as well, or whether the colony-level differences seen in honey bees are instead an emergent property of individual-level cognitive differences similar to what has been found in humans.

Diverse, co-occuring signals can have a variety of effects on an organism. In some cases, a relevant signal must be "extracted" from a milieu of distracting and possibly irrelevant cues (Conchou et al., 2019; Deisig et al., 2014; Gomes & Goerlitz, 2020; McDermott, 2009; Ord et al., 2007). In other cases where multiple cues are involved in mediating a complex process, a second cue can enhance the response to a primary signal, such as when host plant volatiles increase the response to sex pheromones (Schröder & Hilker, 2008). The current study highlights a unique case where both the target cue and the conflicting information are relevant to the organism but have historically been considered the domains of distinct task specialists. Our results suggest that these "specialists" may be paying attention to a broader array of cues than previously appreciated, albeit with colony-level variation related to cue sensitivity. Future experiments should consider how pheromone sensitivity tracks worker bee progression through various temporary behavioral

specializations associated with adult temporal polyethism. Certain cues may be prioritized, not just because of behavioral specialization but because of collective colony-level priorities. The regulation of diverse behaviour in the honey bee nest may be much more nuanced than previously appreciated.

# CHAPTER 4. ANTENNAL SENSITIVITY TO TASK-SPECIFIC PHEROMONES IS AFFECTED BY TASK SPECIALIZATION AND COLONY AGGRESSION IN THE HONEY BEE (*APIS MELLIFERA*).

*Rebecca R. Westwick, Zainulabeuddin Syed, Clare C. Rittschof*

Many animals rely on cues from the environment to make decisions about which behaviors to perform. This can be particularly important for social species, where social signals can help to coordinate behaviors between and among individuals. Sometimes, though, different individuals will respond differently to the same information. One example of this phenomenon can be seen in honey bees. Adult honey bee workers will temporarily specialize on a particular hive task, such as nursing the young or defending the colony. Adults will eventually perform most of the tasks at some point in their life. Each of these tasks rely on different pheromones to coordinate effort within the colony. But for an individual worker bee who is currently specialized on one task, what makes her more responsive to that task's associated stimuli than other cues within the hive? Two primary possibilities included differences in the peripheral nervous system (detection of the signal in the antennae) and differences in the central nervous system (processing of the signal in the brain). We tested whether peripheral (antennal) sensory sensitivity to two social pheromones is associated with task specialization in honey bees. Additionally, we examined how a social factor that is known to influence these behaviors, colony-level aggression, affects these dynamics. Our work reveals a mild association between peripheral sensory sensitivity and certain task specializations, but we also found complex influences of colony-level aggression and intensity of the stimulus on these dynamics.

## 4.1 Introduction

Social signals are important for correctly orchestrating many key behaviors. This is true for dyadic interactions, such as in the contexts of mating and parent-offspring interactions (Capas-Peneda et al., 2022; Charlesworth et al., 2000; Herberstein et al., 2014). But successful coordination is particularly critical for complex, group-level phenotypes (Demartsev et al.). For example, dwarf mongooses use distinct vocalizations to recruit nestmates in different contexts (reuniting the group versus recruitment to mob a predator), and individuals responding to these distinct calls show different, context appropriate behaviors (Rubow et al., 2017). For communication to be effective, the sender must produce the correct signal and the receiver must be able to detect and correctly interpret it (Wiley, 2006). Thus, features of the sensory systems of the receiving organism can have a huge impact on the outcomes of communication.

The first step in the reception of a cue is peripheral detection. The receiver must be able to see, smell, hear, taste, or feel the signal being sent, depending on the modality of the signal. Detection is therefore affected by the strength of the signal as it leaves the sender but also by the local environmental conditions and the sensory sensitivity of the receiving organism (G. L. Cole, 2013). Increasingly, individual differences in sensory sensitivity are thought to contribute to social behaviors such as mate choice and parental care (Ronald et al., 2012). For example, a recent study in honey bees implicated differences in antennal sensitivity to brood pheromones in the level of nursing effort directed towards queen larvae

by selectively-bred nurse bees versus wild-type nurses (Wu et al., 2019). This process of receiving signals, already affected by factors such as individual sensitivity, can be further complicated when there is other information that the organism must contend with and potentially filter out (Oka et al., 2004). Alternative information can cause issues at multiple levels of detection, signal processing, and attention (Rosa & Koper, 2018). A prime example of this phenomenon can again be found in honey bees, which must contend with more than a dozen different types of pheromones that can be found within a hive at a given time.

Honey bees rely in large part on pheromones to coordinate the many in-hive tasks that keep the colony functioning. There are specific pheromones related to most of these tasks, such as brood care (He et al., 2016; Pankiw et al., 1998), tending of the queen (Hoover et al., 2005), foraging (Reinhard & Srinivasan, 2009), nest defense (Breed et al., 2004), and many other functions (Bortolotti & Costa, 2014). Adult worker bees will temporarily specialize on a given task or set of tasks in a well-defined pattern called "age polyethism" (Seeley, 1982). The youngest bees serve as nurses that tend the brood. Middle-aged bees transition to other in-hive tasks, such as comb building and food storage. The oldest bees transition once again to performing out-of-hive tasks, such as guarding the colony entrance and foraging. There is flexibility within this framework, though, and within age cohorts there is individual variation in a worker's proclivity for each task (Calderone & Page, 1988; Robinson et al., 1992; Theraulaz et al., 1998).

All of these different tasks must take place within the colony at all times. Therefore, workers experience competing pressures towards assisting with different colony functions, and different groups of individuals tend to gravitate towards some of these jobs more than

others (e.g. young nurses tend to care for brood, old guards and foragers tend to perform out-of-hive tasks such as food collection and nest defense) (Beshers et al., 2001). We expect nurses to be more responsive to signals related to brood care, such as ocimene, a recently identified putative begging signal (He et al., 2016; Maisonnasse et al., 2009). Meanwhile, we expect guards and foragers to be more responsive to signals related to nest defense, such as the alarm pheromone isopentyl acetate (Robinson, 1987a). It is unclear, however, to what extent these differences in behavioral responsiveness are driven by peripheral sensory sensitivity, higher-order sensory processing, or internally guided cues.

Colony-level phenotypes come into play as well. Some colonies are more defensive than others. These colonies that show a higher propensity for aggressive nest defense are also behaviorally more sensitive to alarm pheromone both at the colony level and at the individual level (particularly individuals of the relevant task specialization) (Alaux et al., 2009; Boch & Rothenbuhler, 1974; Robinson, 1987a). The group-level aggressive phenotype has far-reaching impacts on other aspects of colony health and behavior. Aggressive colonies tend to be healthier and more successful foragers (Rittschof et al., 2015; Rittschof et al., 2019; Wray et al., 2011). Additionally, a recent study identified effects of colony-level aggression on nursing behavior. Westwick et al. 2023 found that nurses from high-aggression colonies decreased larval care behaviors in the presence of alarm pheromone, while nurses from low-aggression colonies did not decrease care. This effect happened independent of any additional effect of the ocimene begging pheromone that we expect would be a primary determinant of nursing effort. Thus, the effects of different pheromones on task performance are complex and multi-faceted, even for tasks these pheromones are not thought to directly regulate, further complicating the picture on

what drives an individual's behavioral sensitivity to these different stimuli. In this study, we examined whether antennal sensitivity to alarm pheromone and ocimene is affected by task specialization and colony-level aggression using electroantennography.

## 4.2 Methods

### 4.2.1 Aggression assays

Honey bee colonies were located on a research farm near Lexington, KY, USA. All colonies were at a mature, full size at the time of collection, were not showing any overt signs of disease, and were not undergoing active mite treatment. Colonies had mostly been established the previous spring as packages advertised as "Italian" and "Russian hybrid." We assayed approximately twenty-five colonies for colony-level aggression following previously established methods (Alaux & Robinson, 2007; Rittschof et al., 2015). Briefly, we took a "before" picture of the front of the hive to get a baseline activity level. We then placed 3uL of 1:10 isopentyl acetate (IPA), an important component of the honey bee alarm pheromone, on a small piece of filter paper at the entrance of the hive. We waited one minute and took an "after" picture. The colony aggression score was calculated as the difference in the number of bees at the entrance of the colony before and after the alarm pheromone was placed. We chose the three highest- and three lowest-scoring colonies for this experiment (N=6 total colonies).

### 4.2.2 Bee collection

All experimental days took place between 15 Sept—7 Oct 2022. Each experimental day would consist of a collection of one type of task specialist from a single colony (e.g. only foragers or only nurses from one colony on a given day). We collected approximately 10 bees per day, with the expectation that we would use 7-8 of them (N=88 total bees across all colonies and treatment groups; unused bees were released back at their colony-of-origin at the end of the day). Collections occurred between the hours of 08h00 and 11h00 (temperature-dependent). To collect foragers, we would place a queen excluder (a grate that is just large enough for an adult worker to fit through) in front of the entrance of the hive. Foragers returning to the hive would land on the queen excluder. We would trap a plastic baggie over each bee and use soft forceps to transfer them to a small, ventilated plexiglass cage provisioned with 50% sucrose ad libitum. To collect nurses, we would remove a frame of open brood from the colony and watch for bees to make "nursing visits" (placing their head and/or body into a honeycomb cell with a larva). Any bees observed to have performed nursing behavior were gently grabbed with soft forceps and placed directly into a similar plexiglass cage. The cage of bees was promptly transferred to an insulated box lined with a damp towel to preserve the humidity in the container and the bees were immediately brought to the laboratory for electroantennography.

### 4.2.3 Odorants

We created serial dilution of both IPA (Sigma, 112674) and ocimene (Sigma, W353901) in mineral oil (Sigma, M8410), as well as pure mineral oil for a control. Dilutions included 1:1, 1:10, 1:100, 1:1000, and 1:10,000. We loaded 20uL of solution

onto individual pieces of filter paper (VWR) that were placed into sterile syringes that could be interchangeably introduced into the airstream of the electroantennogram apparatus. Odorants were refreshed between every 3-4 preparations. The same stock dilutions were used throughout the course of the entire experiment.

### 4.2.4 Antennal preparation

An individual bee would be removed from the plexiglass cage into a plastic baggie and grabbed with a pair of soft forceps. We euthanized the bee via rapid decapitation with a scalpel followed by pithing of the head capsule after the antenna was removed. We clipped off the right antenna (Frasnelli et al., 2010; Letzkus et al., 2006) at the base of the scape with microscissors and immediately dabbed on electroconductive glue (Parker: Spectra 360 Electrode Gel) to prevent desiccation. Under a microscope, we positioned the clipped end of the antenna into a glass electrode that had been pre-filled with Kaissling Ringer solution. The electrodes were constructed from borosilicate micropipettes (World Precision Instruments) that had been stretched on a P-2000 Laser Based Micropipette Puller and filed to an internal diameter slightly larger than the diameter of the antenna. A 1.2mm micropipette was used for the ground electrode and a 1.0mm micropipette was used for the recording electrode. After the ground electrode was placed, we used microscissors to clip off a small section at the distal tip of the antenna and once again dabbed the clipped end with electroconductive glue. The setup was then transferred to a TMC Vibration Isolation table with a microscope (Olympus BX51WI), and we positioned the clipped tip into the pre-filled recording electrode with DC3001R type Motorized Micromanipulators

under the guidance of the microscope. The time elapsed between the recording electrode being placed and euthanasia was typically around 7 minutes.

### 4.2.5 Electroantennography

The recording electrode was connected to a universal single-ended probe to serve as a pre-amplifier that fed into an IDAC-4 4-Channel USB acquisition controller and Syntech CS-55 stimulus controller. These apparatuses were connected to a computer with EAGPro software (Syntech). We used a low-cutoff filter set at 0.1Hz to improve signal-to-noise ratio. Odorants were delivered in a clean, humidified airstream via a Y-tube compensatory flow system. The airspeed was measured at 0.4m/s with a temperature around 23ºC. The continuous airflow was 1L/min with a pulse flow (compensated) of 0.4L/min. and a pulse duration of 0.5 seconds. Recordings were captured for 10 seconds following delivery of the stimulus. Recordings took place between approx. 11h00—16h00. The recording apparatus and odorant delivery system were regularly tested with an antenna signal stimulator and a TA5 anemometer/thermometer to ensure similar conditions across recording days.

### 4.2.6 Stimulus presentation

We began each individual bee's recording session by introducing a test stimulus of 1:10 IPA to ensure that the electrodes were making proper contact (the syringe used for the 1:10 IPA test was separate from the syringe used for the 1:10 IPA recording used in experimental analysis, ensuring that this stimulus did not deplete sooner than the other

prepared odorants). After this test odorant, we would deliver a test of the solvent control, plain mineral oil, one minute later. We would then wait two minutes before beginning the experimental stimulus presentations. Odorants were presented one at a time (IPA or ocimene) in order from lowest concentration to highest concentration, each dose one minute apart, followed by three minutes of no stimuli before repeating the process with the other odorant. The order of which odorant was presented first was randomized for each bee. After both odorants had been presented at all concentrations, we would wait another three minutes and deliver a second test 1:10 IPA stimulus and a second mineral oil control stimulus to ensure that the quality of the recordings did not diminish over the course of the recording timeframe. We saw no significant differences in the response in these pre- or post-experimental test recordings (see Appendix 3, Fig. A3S1). The entire recording session lasted approximately 18 minutes from the presentation of the first stimulus.

### 4.2.7 Statistical analysis

All analyzed antennal responses were normalized by subtracting the average of the two measured mineral oil responses (pre- and post-treatment) from each measurement. Statistical analyses were carried out using R version 4.1.2 (R Core Team, 2021). We used the function wilcox.test::stats for paired Wilcoxon signed-rank tests (R Core Team, 2021). We used the "PairedData" package to create visualizations of paired data (Champely, 2018). We used the lmer::lme4 function to run linear mixed models (Bates, 2015). For multivariate models, we ran them with the lmer function as well using the syntax and procedure outlined by Dworkin and Bolker (Dworkin & Bolker, 2021). All models contained task specialization, colony-of-origin aggression, and their interaction as fixed

effects and Colony ID as a random effect. Model assumptions were checked using the DHARMa package (Hartig, 2022). Significance values were calculated with the Anova::car function (Fox & Weisberg, 2019). Post-hoc tests were carried out with the emmeans package (Lenth, 2022). Plots were made using the packages cowplot, ggplot, GGally, ggpubr, ggsignif, and viridis (Ahlmann-Eltze & Patil, 2021; Garnier et al., 2021; Kassambara, 2020; Schloerke et al., 2021; Whickam, 2016; Wilke, 2020).

## 4.3 Results

### 4.3.1 Signal characteristics

At the three highest odorant concentrations (1:1, 1:10, and 1:100 dilutions, Fig 4.1, C-E), we observed stronger antennal response (maximum depolarization) to isopentyl acetate (IPA, a component of the honey bee alarm pheromone) than ocimene (recently identified as a putative begging signal). For example, at the 1:1 dilution, the mean response (± standard deviation, normalized to the solvent control) to IPA was 0.6 mV (±0.1) while the response to ocimene was only half as strong at 0.3 mV (±0.08; all valued blank-corrected). These differences were statistically significant when assessed with a paired Wilcoxon signed-rank test ($V = 23$, $p = 1.23e\text{-}15$). Similar ratios were observed for the 1:10 and 1:100 dilutions (paired Wilcoxon test: 1:10: $V = 9.5$, $p = 7.72e\text{-}16$; 1:100: $V = 33.5$, $p = 2.612e\text{-}15$). At lower concentrations, however, we observed the opposite: there was stronger antennal response to ocimene than isopentyl acetate (Fig 4.1, A-B). The mean response to ocimene (± standard deviation) at the 1:1,000 dilution was twice as strong (0.02mV (±0.02) for ocimene vs 0.01mV (±0.01) for IPA), and was ten times as strong at the 1:10,000 dilution (0.01mV (±0.02) for ocimene vs 0.001mV (±0.02) for IPA), though

the absolute magnitude of response at this dilution was quite small (paired Wilcoxon test: 1:1,000: V = 2668, p = 0.0014; 1:10,000: V = 3053.5, p = 3.536e-07). Figure 4.2 shows the receptor potential signal traces for each odorant, averaged across all individuals, with the odorant applied at Time=0.5s. Overall, responses were detectable at lower concentrations for ocimene than for IPA (Fig 4.2A versus 4.2B), but signal strength increased more slowly with concentration. Figure 4.3 shows the dose-response curves for each odorant.

Figure 4.1

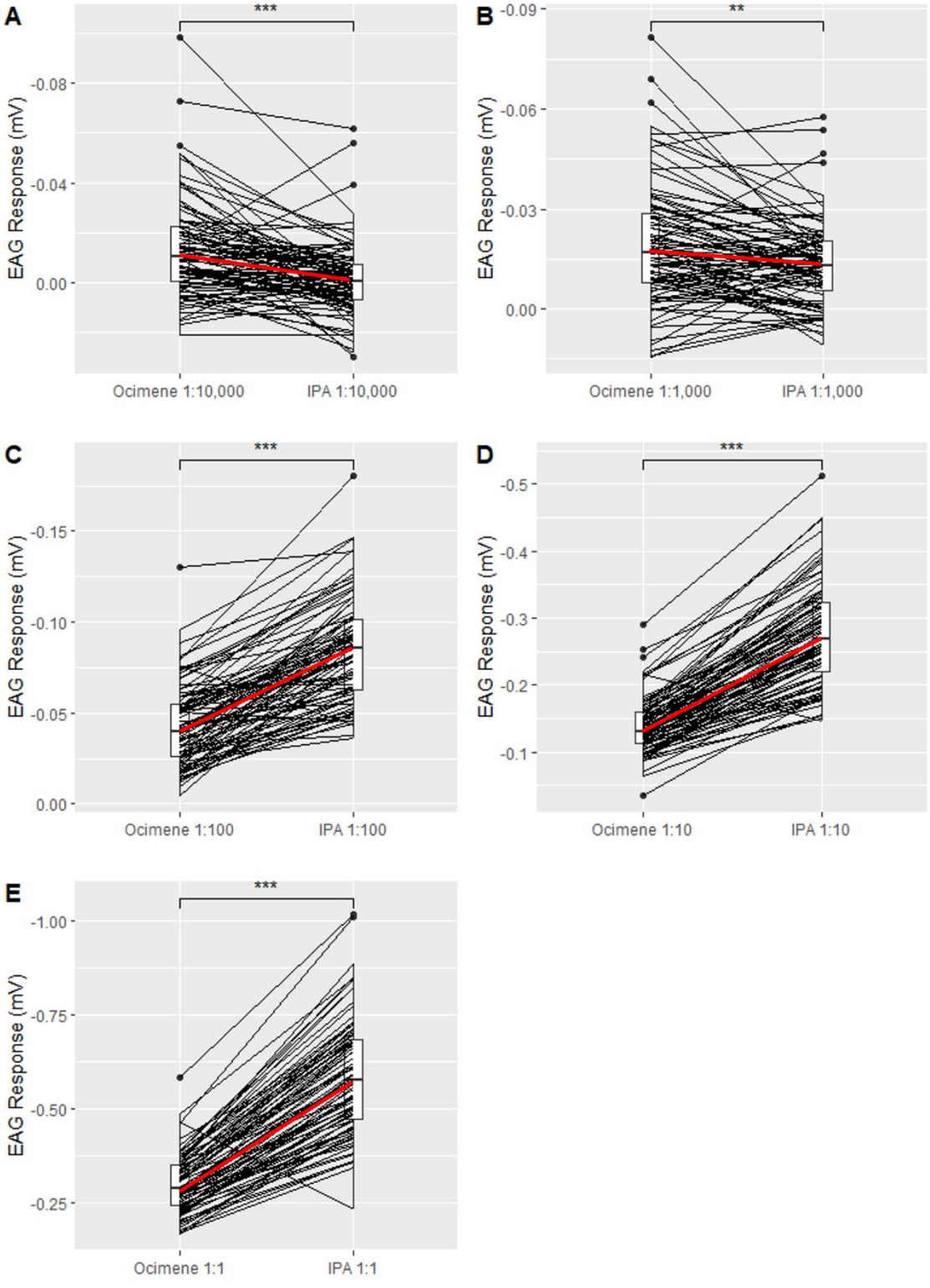

A
***
EAG Response (mV)
-0.08
-0.04
0.00
Ocimene 1:10,000
IPA 1:10,000
B
**
-0.09
-0.06
-0.03
0.00
Ocimene 1:1,000
IPA 1:1,000
C
***
-0.15
-0.10
-0.05
0.00
Ocimene 1:100
IPA 1:100
D
***
-0.5
-0.4
-0.3
-0.2
-0.1
Ocimene 1:10
IPA 1:10
E
***
-1.00
-0.75
-0.50
-0.25
Ocimene 1:1
IPA 1:1

Figure 4.1: Honey bees show stronger antennal response (maximum depolarization) to ocimene (recently identified as a putative begging signal) at low concentrations, but isopentyl acetate (IPA, a component of honey bee alarm pheromone) at high concentrations. Antennal response in millivolts to ocimene and IPA at 1:10,000, 1:1,000, 1:100, 1:10, and 1:1 dilutions (A-E respectively). Each line represents one bee's response to both odorants at matched concentrations. Red lines display median change in response. Significance stars are based on paired Wilcoxon signed-rank tests.

Figure 4.2

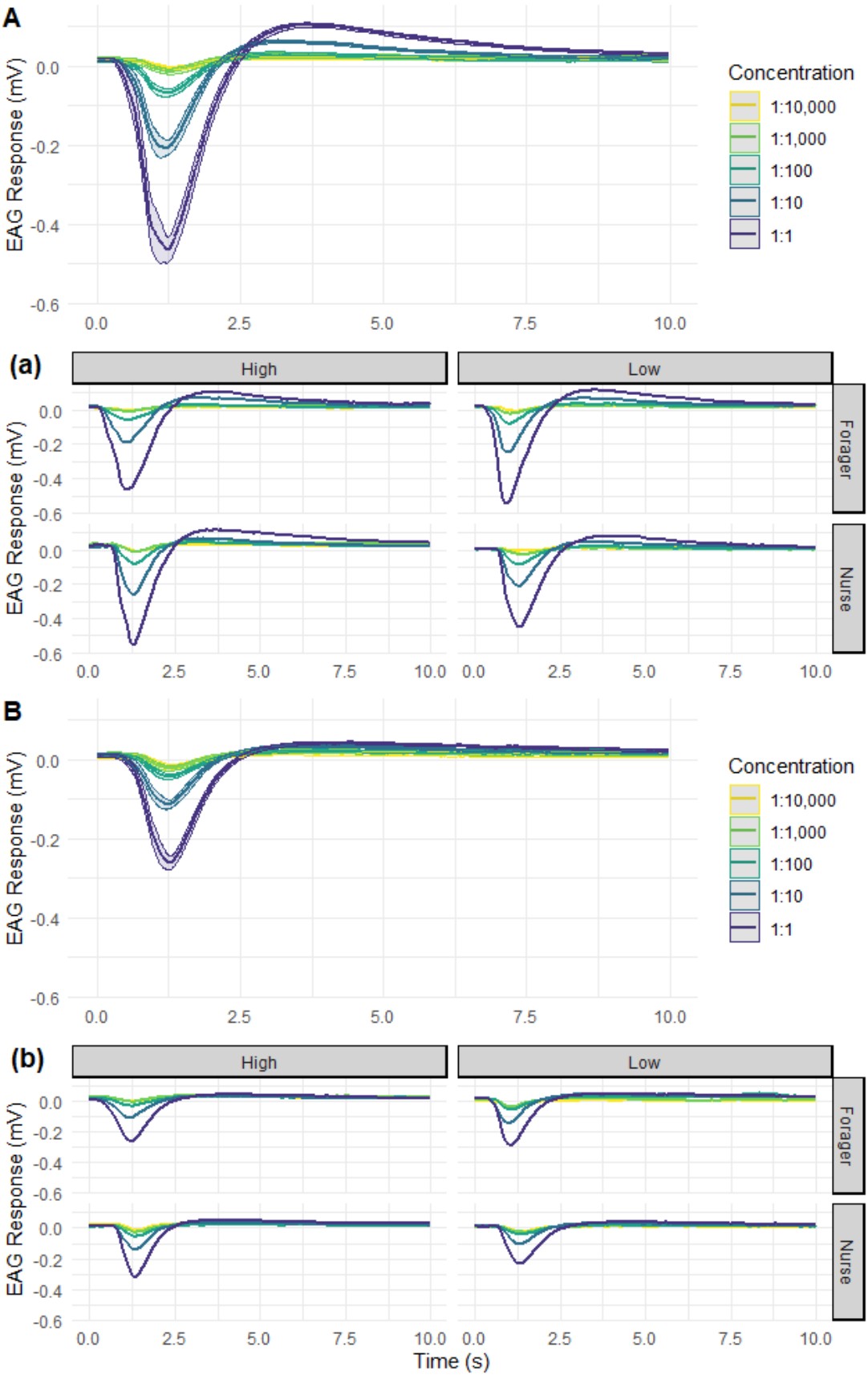

A
EAG Response (mV)
0.0
-0.2
-0.4
-0.6
0.0
2.5
5.0
7.5
10.0
Concentration
1:10,000
1:1,000
1:100
1:10
1:1
(a)
High
Low
Forager
Nurse
B
(b)
Time (s)

Figure 4.2: Honey bees of all task specializations and aggression levels showed stronger antennal response at high concentrations of isopentyl acetate (IPA, a component of honey bee alarm pheromone), while bees showed a proportionally stronger response at low concentrations to ocimene (recently identified as a putative begging signal). Receptor potential signal traces for isopentyl acetate (IPA, "A") and ocimene ("B"). "A" and "B" show IPA and ocimene respectively averaged across all individuals in the study (N=88). Translucent sections around the main trace represent the standard error. "(a)" and "(b)" show the same responses for IPA and ocimene respectively, but they are broken into groups by task specialization and colony aggression level. Each sub-plot within "(a)" and "(b)" shows the averaged responses of 21-23 individual bees from 3 colonies (88 total individuals, 6 total colonies). For all panels, the odorant was applied at 0.5 seconds into the 10-second-long recording and lasted for 0.5 seconds. Each color represents a different concentration of the odorant, with light yellow being the lowest concentration (1:10,000) and dark purple being the highest concentration (1:1).

Figure 4.3

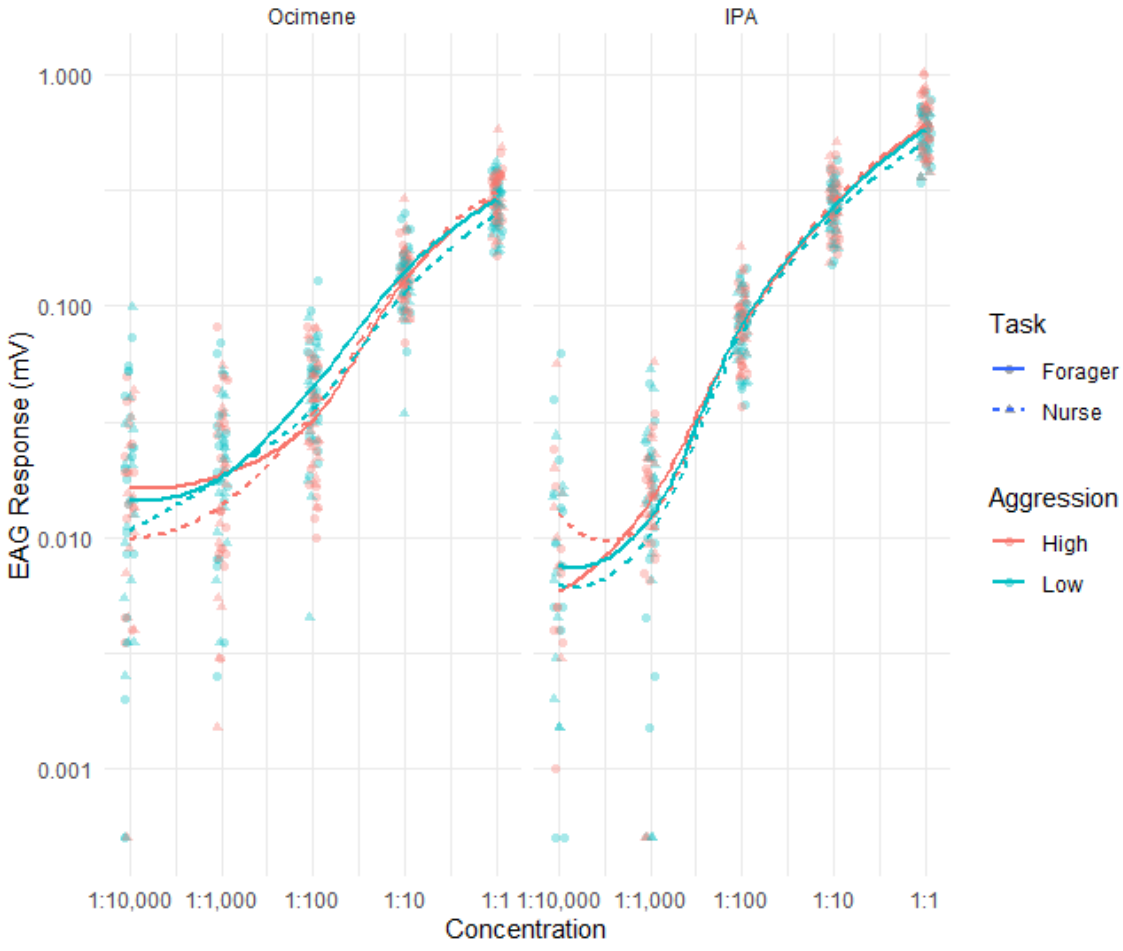


Figure 4.3: Honey bees of all task specializations and aggression levels showed stronger antennal responses (maximum depolarization) to isopentyl acetate (IPA) than ocimene at high odorant concentrations. Dose response curve of isolated honey bee antennae to five concentrations of IPA or ocimene (responses normalized to the solvent control). Each point represents one bee's response. Curves are smoothed conditional means using a loess function. Foragers are circles with solid lines, nurses are triangles with dashed lines. Bees from high-aggression colonies are in red, bees from low-aggression colonies are in blue. Note the log scale on the y-axis.

### 4.3.2 Antennal response to ocimene and isopentyl acetate for different colony aggression levels and task specialists

#### 4.3.2.1 Ocimene

Considering all concentrations, we found that only aggression affected the antennal response to ocimene. We used a multivariate linear mixed modeling approach outlined by Dworkin and Bolker (Dworkin & Bolker, 2021). Our model assessed all five concentrations (1:1-1:10,000) with colony aggression, task specialization, and their interaction as fixed effects and colony ID and individual ID as random effects. Only colony aggression significantly affected antennal response to ocimene overall (Appendix 3, Figure A3S2, bottom left; ANOVA: Aggression: Wald $X^2_5 = 14.4$, $p = 0.01$; Task: Wald $X^2_5 = 3.9$, $p = 0.57$; Aggression*Task interaction: Wald $X^2_5 = 7.2$, $p = 0.21$). Results were similar when analyzed as a MANOVA that did not take colony ID into account.

We additionally examined the antennal response to ocimene specifically at a concentration that has been demonstrated to have behavioral relevance, which for ocimene was 1:10 (Westwick et al., 2023 *in press*). We found that antennal response to ocimene was stronger in nurses from high-aggression colonies than low-aggression ones, while foragers showed no difference based on colony aggression level (Fig. 4.4 top). Using a linear mixed model with Colony ID as a random effect, we found that there was a

significant interaction between colony aggression level and nurse-versus-forager task specialization with neither main effect being significant (ANOVA: Aggression: Wald $X^2_1$=1.29, p = 0.26; Task: Wald $X^2_1$=0.75, p = 0.39; Aggression*Task interaction: Wald $X^2_1$=5.87, p = 0.015). As expected due to the strong correlation between concentrations (Appendix 3, Figure A3S3), results were similar for a linear mixed model on the antennal response to 1:1 ocimene (ANOVA: Aggression: Wald $X^2_1$=0.005, p = 0.94; Task: Wald $X^2_1$=0.69, p = 0.41; Aggression*Task interaction: Wald $X^2_1$=4.50, p = 0.03). Figure 4.2(b) shows the averaged receptor potential signal traces divided by colony aggression and task specialization for ocimene at all five concentrations.

Figure 4.4

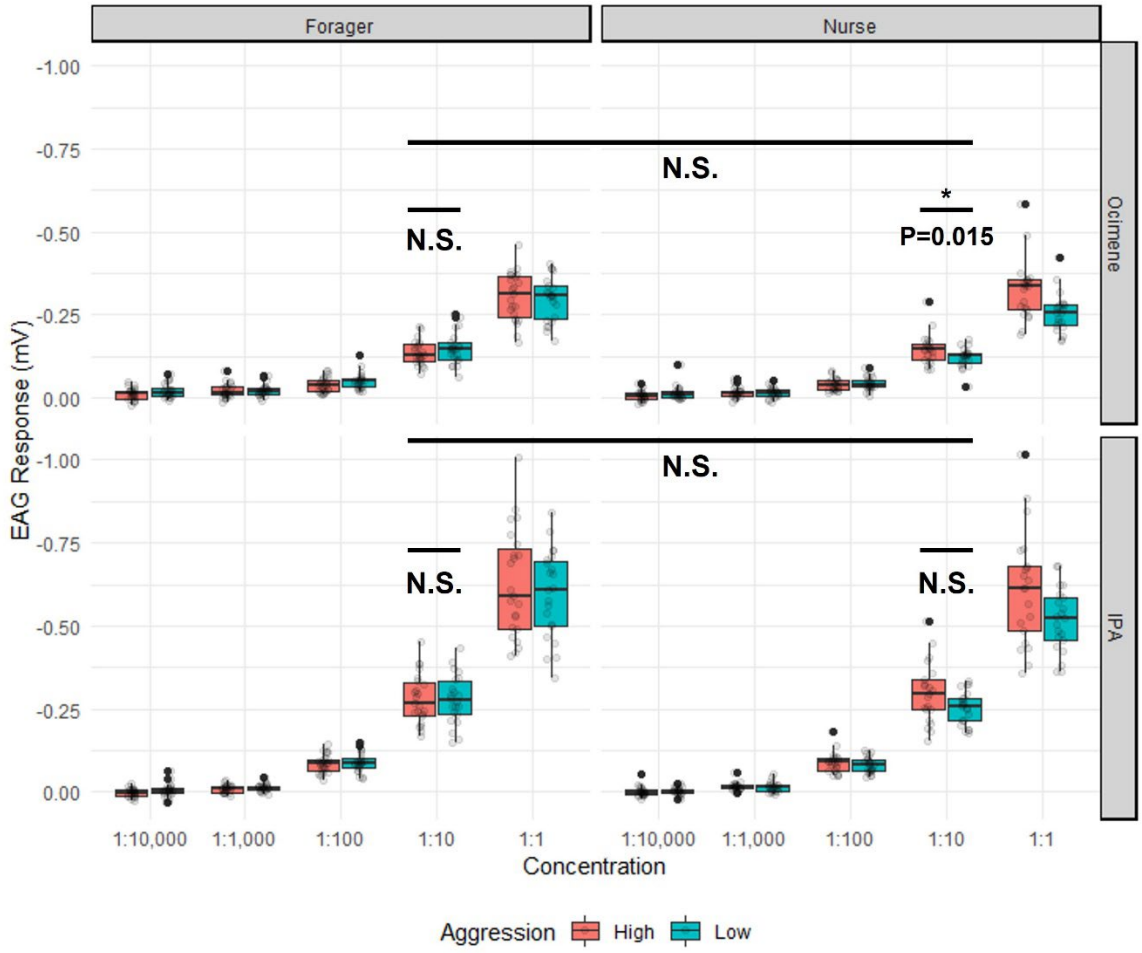



Figure 4.4: (Top) Nurses from high-aggression colonies show a stronger antennal response to 1:10 ocimene (a putative begging signal) than nurses from low-aggression colonies. Foragers show no difference. (Bottom) Neither nurses nor foragers show a difference in antennal sensitivity between colony-of-origin aggression levels for 1:10 isopentyl acetate (IPA, alarm pheromone).

Because we only saw a significant interaction effect, we performed an estimated marginal means post-hoc comparison to assess which sub-groups contained significant differences. We were most interested in the differences between nurses from high- and low-aggression colonies due to previous behavioral results (Westwick et al. 2023), so we first looked at comparisons within task specialization (i.e. between nurses of each aggression level, then between foragers of each aggression level). We found that the

difference between aggression levels was significant within nurses but not within foragers (nurses: t-ratio=2.506, p=0.03; foragers: t-ratio=-0.07, p=0.94). The antennal response (maximum depolarization) of nurses from high-aggression colonies was 25.5% stronger than the response of nurses from low-aggression colonies (a difference of 0.07mV). The antennal responses of foragers from high-and low-aggression colonies were nearly identical, with a difference of only 0.6% (0.002mV) (Fig. 4.4 top). We additionally looked at comparisons within aggression level (i.e. between low-aggression foragers and nurses, then between high-aggression foragers and nurses). We found that the difference between task specialists was significant within low-aggression colonies but not within high-aggression colonies (low-aggression: t-ratio=2.162, p=0.03; high-aggression: t-ratio=-0.831, p=0.41). Foragers from low-aggression colonies showed a 16.0% higher response than nurses from low-aggression colonies (a difference of 0.05mV). Meanwhile, nurses from high-aggression colonies showed a non-significant 5.7% stronger response than foragers from these colonies (a difference of 0.02mV).

#### 4.3.2.1 Isopentyl acetate

When we examined all concentrations of isopentyl acetate (IPA), we found that only task specialization affected the antennal response. We used the same multivariate approach described above for ocimene, with a model that assessed all five concentrations (1:1-1:10,000) with colony aggression, task specialization, and their interaction as fixed effects and colony ID and individual ID as random effects. Only task specialization significantly affected antennal response to IPA overall (Appendix 3, Figure A3S2, bottom right; ANOVA: Aggression: Wald $X^2_5 = 5.0$, $p = 0.42$; Task: Wald $X^2_5 = 12.3$, $p = 0.03$;

Aggression*Task interaction: Wald $X^2_5 = 3.6$, $p = 0.61$). Results were again similar when analyzed as a MANOVA that did not take colony ID into account.

We again examined the antennal response to IPA at a concentration that has been demonstrated to have behavioral relevance, which for IPA was 1:10 (Westwick et al., 2023 *in press*). We found an overall similar pattern for isopentyl acetate (IPA) to the one seen in ocimene, where antennal response to IPA was slightly stronger in nurses from high-aggression colonies than low-aggression ones with foragers showing no difference at high odorant concentrations. This effect, however, was weaker than that seen for ocimene, with a smaller effect size that was not statistically significant (Fig. 4.4 bottom). Using a linear mixed model with Colony ID as a random effect, we found no significant effect of task specialization, colony-of-origin aggression, or their interaction (ANOVA: Aggression: Wald $X^2_1=0.02$, $p= 0.90$; Task: Wald $X^2_1=0.68$, $p= 0.41$; Aggression*Task interaction: Wald $X^2_1=1.81$, $p= 0.18$). Figure 4.2(a) shows the averaged receptor potential signal traces divided by colony aggression and task specialization for IPA at all five concentrations. We ran a similar linear mixed model for IPA at the 1:1 concentration to verify this pattern and received similar results (ANOVA: Aggression: Wald $X^2_1=0.40$, $p= 0.53$; Task: Wald $X^2_1=0.11$, $p= 0.75$; Aggression*Task interaction: Wald $X^2_1=0.87$, $p= 0.35$). As with ocimene, most pairs of concentrations were significantly positively correlated with each other for IPA, particularly within the higher concentrations (as the lowest concentrations showed essentially no signal response at all to this odorant; Appendix 3, Fig. A3S4).

We again performed an estimated marginal means post-hoc comparison to examine the effect sizes and significances between sub-groups for our initial analysis of the response to 1:10 IPA. The difference between aggression levels was not significant within either

task specialization (nurses: t-ratio=1.36, p=0.22; foragers: t-ratio=0.13, p=0.90). The antennal response of nurses from high-aggression colonies was a non-significant 17.8% higher than the response of nurses from low-aggression colonies (a difference of 0.05mV), which was smaller than the difference seen between aggression levels for ocimene. The difference in response between high- and low-aggression foragers was again negligible relative to nurses (1.5%, a difference of 0.004mV) (Fig. 4.4 bottom). The difference between task specialists was also not significant within either aggression level (low-aggression: t-ratio=1.08, p=0.29; high-aggression: t-ratio=-0.82, p=0.41). Foragers from low-aggression colonies showed a non-significant 8.4% higher response than nurses from low-aggression colonies (a difference of 0.02mV), while nurses from high-aggression colonies showed a non-significant 5.9% stronger response than foragers from these colonies (a difference of 0.02mV).

## 4.4 Discussion

We found that the antennal response to ocimene, a recently-identified putative begging pheromone, and isopentyl acetate (IPA), a key component of the honey bee alarm pheromone, showed different signal characteristics in honey bees. Antennal responses were detectable at lower odorant concentrations for ocimene than for IPA. This pattern reversed at higher concentrations, however, with the response to dosage increasing much more quickly for IPA than for ocimene. These features may reflect the biology of the behaviors that are governed by these signals: ocimene is released at relatively low concentrations by larvae, so the nurse bees that tend them need to be able to detect this signal even when it is very weak (He et al., 2016; Noël et al., 2023). Meanwhile, IPA is used to recruit nestmates

for aggressive nest defense. A greater number of individuals releasing alarm pheromone typically signals a greater magnitude of threat to the colony. This situation in turn would require a greater recruitment response from individuals inside the colony to help ward off the threat. Therefore, the rapid acceleration in antennal response as concentration of IPA increases may serve to keep the pace of individual responses in step with escalating defensive needs of the colony.

When considering each odorant at all tested concentrations, we found that antennal response to ocimene was affected by colony aggression level alone. The effect of colony aggression on ocimene can be seen in Appendix 3, Figure A3S2 (Appendix 3, Fig. A3S2, bottom left). Bees from low-aggression colonies show a stronger response to ocimene than bees from high-aggression colonies at low-to-moderate concentrations. At the highest concentrations, though, bees from high-aggression colonies show a stronger response. This pattern, where the slope of the curve is steeper for bees in high-aggression colonies, could allow these bees to better identify larvae that are releasing the most ocimene (which would likely be the larvae that had been starved the longest (He et al., 2016) and therefore needed attention most urgently). Meanwhile, antennal response to IPA was affected by task specialization alone when considering all concentrations. The effect of task specialization alone can also be seen in Appendix 3, Figure A3S2 (Appendix 3, Fig. A3S2, bottom right). Foragers show responses that are consistently slightly stronger than nurses at all concentrations. This pattern is in line with the finding that foragers show a stronger behavioral response than nurses to this pheromone, although the small magnitude of the sensitivity difference is notable.

When considering concentrations with demonstrated behavioral relevancy, we found that nurses from high-aggression colonies were more sensitive to ocimene at the 1:10 concentration than nurses from low aggression colonies. Foragers were equally sensitive to ocimene at this concentration regardless of colony-of-origin aggression. We found a similar pattern for antennal sensitivity to IPA at the 1:10 concentration, though this effect was weaker in magnitude and statistically nonsignificant. Nurses from high aggression colonies may be generally more sensitive to social odors (or all odors) than nurses from low aggression colonies. It is interesting to note that this effect was stronger for the odorant that these individuals are "supposed to be" cuing into (ocimene) based on their task specialization. These findings could play into the pace-of-maturation hypothesis for honey bee aggression. This hypothesis posits that low-aggression bees develop more slowly and are therefore "younger" physiologically than a same-aged honey bee that is more aggressive (Pearce et al., 2001; Robinson, 1985; Winston, 1992). It is known that very young bees have slightly lower olfactory sensitivity, though this effect diminishes within a few days of adult emergence (Robinson, 1987a). The lowered sensitivity typically lasts for around 3 days, while nursing behavior lasts for around 9. It is possible that our nurses from low-aggression colonies were shifted more towards physiological youth, with a greater number of individuals still being in this early period of lowered sensitivity, than nurses from high-aggression colonies. By the time they develop into foragers, though, the low-aggression individuals may have "caught up," since the range of foraging ages does not overlap at all with the period of lowered olfactory sensitivity. This would explain why foragers did not show differences in antennal response based on colony-of-origin aggression level while nurses did.

We chose to collect our individuals in a manner that was focused on behavioral task specialization and was age-blind. This choice was intentional, as we wished to make a direct connection between olfactory sensitivity and behavior rather than physiological age. This choice is also relevant for a natural colony context, where workers show a range of ages within each task specialization (Seeley, 1982). This decision did likely introduce more variation in our results, however. It is possible that we would have seen more robust differences had we collected age-matched individuals (Allan et al., 1987).

Behavioral data from Westwick et al. 2023 showed that nurses from high-aggression colonies changed their larval care behaviors in the presence of alarm pheromone, while nurses from low-aggression colonies did not. Our results showed that nurses from high-aggression colonies had a slightly higher, though statistically nonsignificant, antennal sensitivity to alarm pheromone at the concentration used in that study. These results are in accordance with one another, though the small magnitude of the difference seen in this study (~18%) suggests that other mechanisms (such as processing of the olfactory signal deeper in the brain) may be at play as well. This reasoning is especially relevant given that the high concentration of IPA used in Westwick et al. 2023 makes it unlikely to be a matter of a detection threshold difference between nurses from high- and low-aggression colonies.

Additionally, Rittschof et al. 2015 found that larvae that were raised in high-aggression colonies grew up to be more aggressive than larvae raised in low-aggression colonies, independent of proximate adult environment or genetic differences (Rittschof et al., 2015). When larvae are given a short-term nutritional stress late in development, they go on to show increased starvation resilience in adulthood (Wang, Campbell, et al., 2016;

Wang, Kaftanoglu, et al., 2016). They additionally show changes in juvenile hormone titers and glycogen stores as adults, both of which have notably been associated with increased aggression in other studies of honey bees (Pearce et al., 2001; Robinson, 1987a, 1987b). Our study shows that bees from high-aggression colonies are less sensitive to ocimene at low to moderate concentrations and more sensitive at high concentrations than nurses from low-aggression colonies (Appendix 3, Fig. A3S2, bottom left). It is possible that the nurses from high-aggression colonies therefore do not respond to larvae that are in the early stages of starvation, leading to the adaptive effects seen in short-term nutritional stress and higher aggression in adulthood, but then respond more strongly once the larvae begin to reach dangerous levels of food deprivation and before mortality effects set in. A follow-up study could explicitly test this possibility.

It is interesting to note that our field assay to determine which colonies were high- versus low- aggression involved behavioral response to IPA. Our results here, however, showed that individual foragers from each type of colony have essentially no difference in antennal sensitivity to this odorant. This finding suggests that variation in individual and colony level response to alarm pheromone may be guided by other factors, such as differences in higher-order brain centers, individual internal state, or other social or colony-level factors.

Understanding how different individuals parse competing cues can be difficult, particularly when those cues can have effects beyond the behaviors they are primarily intended to regulate. For example, honey bee behavioral task progression involves a shift in which behaviors a bee performs and therefore which pheromones are most relevant to her immediate work. It is known that this task progression is affected by physiological

factors such as age and hormonal status (Pearce et al., 2001; Robinson, 1987b; Seeley, 1982). However, it is not well understood how these changes would manifest within the sensory and neural structures that organize these behaviors. Our work has found evidence for a limited role of peripheral sensory sensitivity in driving these changes. We found over all concentrations tested, isopentyl acetate (IPA), an important component of the honey bee alarm pheromone, elicited a stronger antennal response in the task specialists that are expected to prioritize this signal (foragers). On the other hand, we did not find significant differences in the antennal response of different task specialists to ocimene, a signal related to brood care, over all concentrations tested. This picture is further complicated by social factors, however; the antennal response to all concentrations of ocimene was affected by colony aggression level, despite the fact that aggressive behavior is more typically associated with alarm pheromone. The concentration of the odorant also proved relevant; at a specific concentration of ocimene that has been used in previous behavioral studies, aggression and task interacted in complex ways to affect antennal response. Future studies could probe the antennal response of bees of different task specializations and aggression levels when presented with these two pheromones simultaneously in different ratios, as this may elucidate some of these complex patterns. In sum, differences in peripheral sensory sensitivity may have a limited role in immediately driving an individual's propensity to respond to task-specific stimuli, but these differences are likely not acting alone. Sensory differences probably in concert with other differences, such as changes to higher-order sensory processing centers of the brain to bring about the complex trade-offs between competing task pressures in these insects.

# CHAPTER 5. ALLOGROOMING ALTERS THE EXPRESSION OF GENES RELATED TO IMMUNITY AND SOCIAL RESPONSIVENESS IN THE WESTERN HONEY BEE (APIS MELLIFERA)

*Rebecca R. Westwick, Anna M. Foose, Clare C. Rittschof*

Social behavior and disease mitigation have co-evolved in complex ways in social organisms. Allogrooming is a behavior that serves a direct function of parasite and pathogen removal but has also been co-opted to serve important social functions. Furthermore, allogrooming is a prosocial behavior that comes with potential risks to an individual that performs it, such as the risk of disease spread if the individual they are grooming is infected with a pathogen. These complex interactions can make it difficult to interpret the coevolutionary forces that act on these behaviors. In this study, we investigated whether gene expression links the social and immune components of allogrooming behavior in the Western honey bee (*Apis mellifera*). We found that both performing and receiving allogrooming affect the expression of immune genes (*argonaute-2* and *PGRPSC4300*) in the fat body, an immune and endocrine organ. In addition, performing and receiving allogrooming both alter the expression of genes that have recently been implicated in social responsiveness in the brain (*ftz-f1* and *Nup98-96*). These results suggest that there are direct health benefits to recipients of allogrooming, that potential costs may be mitigated for individuals who perform it, and behavior and immunity are linked through simultaneous action of transcriptional pathways in multiple tissues.

## 5.1 Introduction

Behavior, the brain, and the immune system are inextricably linked in a system called the "neuroimmune axis" (Quan & Banks, 2007). Inflammation can cause wide-ranging effects on components of the nervous system, from dysfunction of glial cells to abnormalities in biogenic amine signaling systems (Felger & Treadway, 2017; Haroon et al., 2017). Inflammation and immune stress cause important changes in behavior in a diseased individual. For example, cytokines induce a suite of adaptations broadly termed "sickness behavior" that are thought to promote recovery, which often involve changes in sleep, appetite, pain, concentration, and reduced engagement with conspecifics (Kelley et al., 2003). The phenomenon of sickness behavior has been identified across a broad range of animal species, including mammals, birds, reptiles, amphibians, fish, and insects (Kazlauskas et al., 2016; Lopes et al., 2021). While some of the mechanisms giving rise to sickness behavior may be broadly conserved, others are unique to certain animal groups depending on the specifics of immune system function and the ways in which immune and inflammation processes interface with the brain (Siddiqui & Khan, 2020; Tizard, 2008).

Behavioral responses to sickness are further elaborated in social species. In these species, social interactions are critical to survival (Snyder-Mackler et al., 2020). But they also increase disease risk, giving rise to complex evolutionary relationships between immune function and behavior (Theis et al., 2015). Social behaviors have been shown to alter immune function (Eisenberger et al., 2017). For example, social isolation increases inflammation and disease risk, while social touch has been shown to reduce stress and enhance immune activation (Hahn-Holbrook et al., 2013; Ironson et al., 1996; Morhenn et al., 2012; Valtorta et al., 2016). These social behaviors go on to impact the immune system

in indirect ways, as they are frequently attached to stress response (Burkett et al., 2016; Morrison, 2016). Conversely, elevated disease pressure due to the ease with which an infectious agent can spread between groupmates has led to the evolution of a behavioral repertoire that mitigates disease transmission (Nunn et al., 2015). "Social immunity" phenotypes are social adaptations that combat disease spread within a group. Common phenotypes include social fever, application of antimicrobial compounds to the nesting site, removal of pathogens and parasites from the nest, and allogrooming (Christe et al., 2003; Cremer et al., 2007; Hart, 1990; Hart & Hart, 2018; Hillegass et al., 2010; Nunn et al., 2006; Starks et al., 2000). Interestingly, social immunity phenotypes are so integral to survivorship that they are tied to other aspects of social living that benefit participants. In group-living animals, disease mitigation behaviors that evolved to serve direct functions (such as parasite or pathogen removal) are often co-opted for social bonding functions (Wilson et al., 2020). For example, allogrooming—cleaning and removing ectoparasites from social partners—is critical for social bonding in many species (Kenny et al., 2017; Matheson & Bernstein, 2000; Russell & Phelps, 2013; Sato et al., 1993). Allogrooming can incur costs, however, such as the risk of the groomer becoming ill if the recipient of the allogrooming is infected with a pathogen (Theis et al., 2015). As a result, disease-related behaviors in social species carry both risks and benefits for the performer and recipient.

In this study, we examined if performing a risky prosocial behavior, allogrooming an infected groupmate, alters the expression of immune genes that could alleviate the effects of infection for the groomer or the recipient. We additionally examined whether performing this behavior alters the expression of genes associated with social

responsiveness as a way to promote social cohesion within the group. We sought to answer two overarching questions: 1) Does the act of performing allogrooming have protective effects on the groomer? and, 2) Does allogrooming have indirect effects on the immune system and other positive social effects, as social immunity phenotypes do in other species? We chose to examine these questions in the western honey bee, *Apis mellifera*. Honey bees live in large, dense, enclosed nests of thousands of individuals where the risk of disease spread is large (Betti et al., 2014). Honey bees have therefore evolved a complex set of social immunity phenotypes to lessen this risk, including frequent allogrooming (Cremer et al., 2007; Simone-Finstrom, 2017). Honey bees have been shown to increase allogrooming towards a nestmate who is experiencing an immune challenge relative to uninfected nestmates (Carr et al., 2020). Additionally, honey bees that are behaviorally aggressive both allogroom sick nestmates more frequently and show improved response to immune challenge (Carr et al., 2020). Honey bee workers who allogroom their nestmates more frequently have furthermore been shown to perform better on bacterial clearance tests, although the exact mechanism remains unclear (Cini et al., 2020).

We induced pairs of worker honey bees to perform allogrooming, focusing on cases where an uninfected bee groomed a nestmate that had been given a yeast injection. This treatment provokes an immune response in the bee that is injected, but it does not cause her to become contagious to bees that interact with her the way a natural pathogen would (Carr et al., 2020). To examine the social and immunological effects of the allogrooming interaction, we then used qPCR to compare the expression of five immune genes in the fat body (an immune and endocrine organ) and two genes implicated in social responsiveness

in the brain relative to injected and non-injected controls that were not involved in allogrooming interactions.

We measured the expression of five genes in the fat body and two genes in the brain. Our immune-related target genes in the fat body were *defensin-1*, *hymenoptaecin, argonaute-2, hopscotch,* and *PGRPSC4300*. These target genes cover a variety of immune and disease response mechanisms in honey bees, allowing us to assess several potential ways that allogrooming could affect the immune system. *Defensin-1* and *hymenoptaecin* encode antimicrobial peptides, which are particularly important for antibacterial and antifungal defense (Danihlík et al., 2015). *Argonaute-2* is a gene encoding a protein involved in the RNA-interference process, which is a key component of antiviral immunity in honey bees (Galbraith et al., 2015). *Hopscotch* is an important gene in the Jak/STAT pathway, which encodes other antimicrobial effector molecules and has shown to be affected by fungal *Nosema ceranae* infections, ingestion of fungicides, and viral IAPV infections in honey bees (Chen et al., 2014; Glavinic et al., 2019). This pathway is also seen in mammals and is implicated in inflammation and wound repair (Myllymäki & Rämet, 2014). And finally, *PGRPSC4300* encodes peptidoglycan recognition protein S1, part of the pathogen-associated molecular pattern (PAMP) recognition system that is important for initially detecting pathogens in the body and provoking an immune response (PGRPSC4300 activates the Toll pathway that creates Defensin antimicrobial peptides) (Evans et al., 2006a; Larsen et al., 2019). We chose these genes to cover a broad range of possible immune outcomes, from pathogen recognition to effector molecules and intermediates in between. We would not expect all of these genes to be directly affected by our yeast treatment. Some, such as *defensin-1,* and *hymenoptaecin,* have antifungal

properties and have previously been shown to be affected by yeast treatments (Carr et al., 2020). *Hopscotch* expression has not been demonstrated to change with yeast treatments to our knowledge, but it is involved with antifungal defense in other contexts (Glavinic et al., 2019). *Argonaute-2* and *PGRPSC4300,* on the other hand, are not directly related to antifungal defense and (as far as we are aware) have not been tested in response to a yeast treatment. However, this does not preclude the possibility that the expression of these genes is affected by allogrooming behavior, so we chose to include them as part of a robust panel examining the effects of this behavior on immune gene expression more generally.

Our genes of interest in the brain were *ftz-f1* and *Nup98-96*. These two genes were identified in previous research as being important for social responsiveness across multiple social contexts in honey bees (Chandrasekaran et al., 2011; Shpigler, Saul, Corona, et al., 2017; Shpigler, Saul, Murdoch, et al., 2017; Shpigler et al., 2018). Bees that were more likely to respond to both a social challenge (defending the group against an intruder bee) and a social opportunity (tending a queen larva) showed differential expression of many genes, and these two showed some of the strongest differences. *Ftz-f1* has additionally been implicated in behavioral responsiveness towards conspecifics in a distant taxon, mice, further motivating its inclusion in this study (Grgurevic et al., 2008). We included these genes in our study to allow us to assess the possibility that participating in allogrooming interactions has social consequences in honey bees, as social benefits of this behavior have been demonstrated widely in vertebrates (Beery & Francis, 2011; Dunbar, 2010; Grueter et al., 2013). If the entanglement of social and immune phenotypes is fundamental to sociality, then we would expect to see these patterns broadly across vertebrates and invertebrates (McFarland; Nuotclà et al., 2019).

## 5.2 Methods

### 5.2.1 Bee sources

Behavioral assays were carried out at the University of Kentucky's Spindletop Farm just north of Lexington, KY, USA on August 28-29, 2020. We chose four colonies from which to collect bees for our experiment. All four colonies had been installed the previous spring from packages advertised as "Russian Hybrid" strain (Schoolhouse Bees, Covington, KY, USA). Two of the four colonies still had their original queen, while the other two colonies had naturally re-queened with a daughter of the package queen. We collected ~30 returning foragers at the entrance of each colony at approximately 0900h. Foragers from each colony were kept in separate ventilated plexiglass cages and supplied with 50% sucrose *ad libitum*. The bees were taken to a covered area a short distance away from the hives for the behavioral assays.

### 5.2.2 Yeast treatment

We pre-prepared a stock suspension of dehydrated *Saccharomyces cerevisiae* (Dadant & Sons, Frankfort, KY, USA) in 10% autoclaved glucose as a growth medium. The suspension was placed in an incubator at 33ºC for at least 24 hours. The night before behavioral assays began, we took ~20mL of the stock suspension and diluted the yeast cells to an OD600 of 0.565 (~$10^9$ cells/mL) in bee saline (Yang & Cox-Foster, 2007). On the day of behavioral assays, we transferred bees in groups of four or five from their plexiglass cage to a plastic bag. Each group was anesthetized in a refrigerator (~3ºC) for 5-10 minutes to sedate them. Once the bees were immobile, we haphazardly selected two bees from each group to be in the "infected" condition and receive a yeast injection. These individuals

were held with forceps and viewed under a dissecting microscope. We used a 30-gauge, 50-uL hand-injector syringe (Hamilton Company, Reno, NV, USA) to inject 2uL of the yeast solution under the 3rd abdominal tergite. Two of the remaining bees were chosen to be in the "uninfected" control condition. These individuals were held with forceps and handled under the microscope in a similar way to the infected bees, but they were not given an injection. We chose not to additionally include a saline injection control, as puncturing the cuticle is known to induce an immune response, and we were most concerned with comparing any immune response (regardless of source) to baseline control (Carr et al., 2020; Yang & Cox-Foster, 2007). Each individual was given a unique mark of Testors model paint (Rockford, IL, USA) after handling that corresponded to its infection status in order to blind the behavioral observers (see below). Each bee was placed in an individual plastic bag with a tube of 50% sucrose until she fully recovered from the cold anesthesia ("recovery" defined as a bee standing upright and moving normally; this process never took longer than 5 minutes post-injection).

### 5.2.3 Behavioral observations and sample collection

The goal of our behavioral observations was to identify and collect four categories of bees for gene expression analysis: uninfected bees that performed allogrooming, infected bees that received allogrooming, and control bees that that were infected and uninfected but did not participate in allogrooming interactions (Table 5.1). This design allowed us to focus on four key comparisons: 1) uninfected control vs. infected control, the effect of our yeast treatment alone (does our yeast treatment provoke changes in the target gene on its own?) 2) uninfected control vs. uninfected groomer, the effect of performing

allogrooming alone (does performing allogrooming change expression of the target gene on its own?); 3) infected control vs. infected recipient, the effect of receiving allogrooming alone (does receiving allogrooming change expression of the target gene on its own?); and 4) uninfected control vs. infected recipient, the combined effects of yeast treatment and receiving allogrooming (how does an infected individual that receives allogrooming look compared to a healthy bee?). We did not collect infected bees that performed allogrooming nor uninfected bees that received allogrooming because these groups were least relevant to our hypotheses, and we did not have the funds to include this larger sample set.

Allogrooming behavior typically involves one individual rubbing its proboscis, mandibles, and sometimes forelegs along the legs, head, side of the abdomen, base of the wings, or antennae of the other bee; the groomer often softly taps her antennae on the receiving bee while doing so. The movements of the groomer are typically much slower and gentler than in other interactions (such as aggressive biting) and the receiving bee typically remains stationary (Carr et al., 2020; Kuswadi, 1992).

We set up pairs of bees for behavioral observations once all four bees in a group had recovered from anesthesia. Two observers would work at the same time, each observing a pair of bees (one infected and one uninfected per pair). Observers were blind to the infection status of each bee (see above), with each observer performing the blinding for the other person. The observers would gently remove one bee from bag where it recovered from anesthesia and place it in the bag with its partner; we haphazardly chose which bee was moved. Each observer then monitored their pair for the first occurrence of allogrooming. As soon as any allogrooming interaction was observed in either pair, the observer would check with the person who placed the paint marks to determine if the

allogrooming interaction occurred in the expected direction—the uninfected bee groomed its infected partner. If the direction was correct, both observers would immediately use forceps to separate their two bees into individual plastic bags again (each still provisioned with 50% sucrose) to prevent further interactions. Thus, of the two pairs of bees observed in a round of the experiment, the allogrooming pair yielded the uninfected groomer and the infected recipient, and the second pair, which was separated before any allogrooming occurred, yielded the uninfected and infected non-grooming controls. We separated both pairs of bees after one allogrooming interaction occurred so that we could isolate the effects of a single, acute event on gene expression (i.e. without risking interference from other social or behavioral effects that occurred if the allogrooming pair had been left together). The time between yeast treatment, behavior, and tissue preservation is described below ("*Timing*"). Individuals from successful rounds would remain in their separate bags for 70 minutes to allow gene expression changes to occur (Carr et al., 2020; Shpigler, Saul, Corona, et al., 2017). Then each bee was flash-frozen in a slurry of dry ice and ethanol, placed into an individual tube, and stored at -80ºC until molecular analyses occurred.

If the direction of the grooming pair was opposite (an infected bee groomed the uninfected partner), all bees four bees from that round would be euthanized via pithing and the data excluded. A round would also be terminated and the bees euthanized if no allogrooming had occurred within 15 minutes of the bees being placed together; however, in all but one round, an allogrooming interaction occurred in one of the two bags within 4 minutes.

### 5.2.4 Timing

The overall timing of a typical round was as follows: bees would be removed in groups from the Plexiglass cage and placed in a refrigerator for anesthesia for approximately 5-10 minutes. We then removed the bees from the refrigerator and performed the yeast treatment, handling control treatment, painting, and placement in individual recovery bags. This process typically took around 4-7 minutes to process all four bees. The bees were then given time to fully recover from the cold anesthesia, which never lasted longer than 5 minutes. Next, we placed the bees together and began behavioral observations. Previous studies (including ones performed in our lab) have found allogrooming to be relatively uncommon in small dish assays; thus, we had anticipated that it would take 10-15 minutes before most grooming interactions would occur (Carr et al., 2020). This would have given us a total time between yeast treatment and behavior of approximately 20-30 minutes and a total time between yeast treatment and flash-freezing of approximately 90-100 minutes. Instead, though, our experiment found allogrooming interactions to be relatively common and to occur very quickly; all useable rounds had an allogrooming interaction occur within four minutes. Thus, our experiment yielded actual times that were closer to 10-15 minutes for the total time between yeast treatment and behavior and 80-85 minutes for the total time between yeast treatment and flash-freezing. The time between behavior and flash-freezing was fixed at 70 minutes. An additional timing factor was that of separating the bees once an allogrooming interaction occurred in one of the pairs: the ease of manipulating the bees within the plastic bag made it so that this step was nearly instantaneous (on the order of a few seconds) once the observer had confirmed with the unblinded individual that the behavior had occurred in the desired

direction. We therefore did not experience problems with the "control bag" allogrooming before we were able to separate the bees.

This timing setup raises the question of whether the uninfected groomers would be able to detect the infection on the infected recipients this quickly, and indeed, if the infected bees' bodies would be able to show a meaningful response within this timeframe. We know from previous studies that the effects of a yeast injection on the production of *defensin-1* and other immune-related molecules in fruit flies occur very rapidly. Elevated expression is detectable within 10 minutes and continues to rise from there (Di Prisco et al., 2013). Therefore, it is quite possible that our 10-15 minute timeframe between yeast treatment and behavior was sufficient to cause a detectable change in the bodies of the infected individuals, putatively through olfactory cues (Conroy & Holman, 2022). Most of the genes in this study have not had detailed time-course analyses conducted regarding the optimal time to measure expression, particularly across the different tissues we are measuring; however, our overall timing of 70 minutes between behavior and freezing and 80-85 minutes between yeast treatment and freezing is well within typical ranges used by other studies for similar genes and contexts (Carr et al., 2020; Di Prisco et al., 2013; Shpigler, Saul, Corona, et al., 2017).

We repeated this process over the two experimental days until we had obtained a sufficient sample size for each treatment group and from each colony (see below, "*Dissection and Gene Expression Analyses*," for details on sample sizes). Any unused bees left over in the plexiglass cages at the end of each day were released back to their colonies.

### 5.2.5 Dissection and gene expression analyses

To obtain fat body tissue, we removed the abdomen of a frozen specimen and partially thawed it in dry-ice-chilled RNAlater ICE (ThermoFisher Scientific, Waltham, MA, USA). This process allowed us to keep the abdomen cold enough to prevent the sample from degrading but thawed enough to be pliable for the dissection. We then removed the gut contents and sting apparatus from the abdomen under a microscope and saved the cuticle, as the fat body tissue adheres to the abdominal wall (Carr et al., 2020). To obtain brain tissue, we removed the head of a specimen and freeze-dried it at -80ºC in a Labconco freeze dryer (Labconco Corporation, Kansas City, Missouri, USA) for approximately one hour. We then placed the head in a shallow bath of 70% ethanol in a dissection dish chilled on dry ice. We cut the top of the cuticle, gently lifted the brain out of the head capsule, and carefully scraped off all glandular tissue from the brain tissue. Following dissection, fat body and brain tissue were stored at -80ºC until homogenization and RNA extraction.

We homogenized individual tissue samples using a FastPrep 5G bead homogenizer (MP Biomedicals, Santa Ana, California, USA) with 5mm stainless steel beads (MSE Supplies LLC, Tucson, AZ, USA). We performed RNA extraction with an E.Z.N.A HP Total RNA Kit with an on-column DNAse treatment (Omega Bio-Tek, Norcross, GA, USA). We then used a Nanodrop Microvolume Spectrophotometer (ThermoFisher Scientific) to determine RNA concentrations. We synthesized cDNA from 200 ng RNA with a SensiFAST cDNA Synthesis Kit (Bioline, Taunton, MA, USA). We then performed qPCR using 10-uL reactions on a 384-well plate with PerfeCTa SYBR Green SuperMix (Quantabio, Beverly, MA, USA). We created a standard curve with genomic DNA diluted

serially four times at 10x dilution intervals. All samples and standards were run in triplicate and with non-template controls on a Quanta Studio 6 (ThermoFisher Scientific).

We measured the expression of five genes in the fat body and two genes in the brain. In addition, we measured two endogenous control genes in each tissue. Our immune-related target genes in the fat body were *defensin-1*, *hymenoptaecin, argonaute-2, hopscotch,* and *PGRPSC4300*. Our endogenous control genes in the fat body were *GAPDH* and *tropomyosin*, both of which have been used as endogenous controls in previous honey bee studies (Carr et al., 2020; Corby-Harris et al., 2019; Jeon et al., 2020). Both genes showed no differences in expression among treatment groups or colonies, so we chose to average their expression and normalized our target genes to this average (Jeon et al., 2020; Lourenço et al., 2008; Vandesompele et al., 2002). Our genes of interest in the brain were *ftz-f1* and *Nup98-96*. Our endogenous control genes in the brain were *GAPDH* and *rps-5a*, which also have previously been used for this purpose (Han et al., 2015; Harrison et al., 2019; Jeon et al., 2020; Manzi et al., 2020; Rittschof, 2017). These brain genes similarly showed no differences in expression among treatment groups or colonies, so we averaged their expression and normalized our genes-of-interest to this average. All primers were ordered from Integrated DNA Technologies (Coralville, IA, USA). Most of our primer sequences were obtained from previous research, but we designed a novel primer for *Nup98-96* using the NIH's primer designing tool (Ye et al., 2012). Primer sequences for all genes analyzed in this study as well as their source can be found in Appendix 4 (Appendix 4, Table A4S1).

Our target sample size was 8-10 samples per treatment group per tissue (N=32-40 total samples per tissue). We were able to achieve this sample size for the brain samples

(Table 5.1). Unfortunately, due to a mechanical failure during tissue homogenization for RNA extraction, our sample size for the fat bodies was below this target (Table 5.1). Our samples were processed in a balanced order, so we do not expect there to be any bias in which samples were lost during this process.

Table 5.1 Table displaying the sample sizes of each tissue type per treatment group.

| | Performed allogrooming ("Groomer") | Received allogrooming ("Recipient") | Non-behaving control ("Control") |
|---|---|---|---|
| Received yeast injection ("Infected") | | Fat body: 5; Brain; 8<br>"Infected Recipient" | Fat body: 7; Brain: 8<br>"Infected Control" |
| No yeast injection ("Uninfected") | Fat body: 8; Brain 9<br>"Uninfected Groomer" | | Fat body: 8; Brain 9<br>"Uninfected Control" |

### 5.2.6 Statistical analysis

All analyses were performed in R version 4.1.2 (R Core Team, 2021). We used the chisq.test() function from the "stats" package to perform a chi-square goodness-of-fit test to assess the direction of allogrooming behavior between infected and uninfected individuals (R Core Team, 2021). All molecular results were analyzed with linear mixed models. For each gene, we began with a model assessing that gene's expression level normalized to the appropriate endogenous control genes (as noted above under "Dissection and Gene Expression Analyses"), log-transformed as necessary (noted where relevant under RESULTS), that included both allogrooming behavior (groomer, recipient, non-

behaving control) and infection status (infected, uninfected) as fixed effects and colony ID as a random effect. These models were built using the lmer() function from the "lme4" package (Bates, 2015). Model diagnostics were assessed using the "DHARMa" package and included a QQ plot, a KS test, a dispersion test, an outlier test, a residual plot, a quantile deviation test, and a combined adjusted quantile test (Hartig, 2022). We then used the AIC() function from the "stats" package to determine whether each model was better fitted with both behavior and infection status, behavior alone, or infection status alone (R Core Team, 2021). We did not include an interaction term in any of our models due to our design not being full-factorial. We used the model with the lowest AIC value for the final analysis. We used the Anova() function from the "car" package to obtain significance values for each fixed effect (Fox & Weisberg, 2019). We used the DunnettTest() function from the "DescTools" package to perform pairwise post-hoc comparisons on models with a significant or trend result (Signorell, 2023). All figures were made with the "cowplot," "ggplot2," "ggpubr," "ggsignif," and "viridis" packages (Ahlmann-Eltze & Patil, 2021; Garnier et al., 2021; Kassambara, 2020; Whickam, 2016; Wilke, 2020).

## 5.3 Results

We observed pairs of honey bees for allogrooming behavior, one of which had been given an injection of yeast to provoke an immune response. Out of the 26 rounds we observed (52 total pairs of bees, see "METHODS"), only one round occurred where no allogrooming was observed in either pair within 15 minutes. Allogrooming tended to occur very quickly once the bees were introduced to the same enclosure, with the mean time to the first allogrooming event occurring approximately 1.8 ± 1.5 minutes after introduction to the enclosure during successful rounds. Allogrooming occurred in the direction we

desired for our molecular analysis (an uninfected bee grooming her infected cagemate) 50% more often than the reverse (15 correct, 10 incorrect; Figure 5.1A). This number was not statistically significant when assessed with a chi-square goodness-of-fit test, possibly due to the small sample size used in this study ($\chi_1$=1, p=0.32). We also note that this effect was inconsistent across bees from different colonies; three of our colonies performed allogrooming in the desired direction well over the 50% chance threshold, while one colony was below (Figure 5.1B). Previous research showed that the aggression level of a colony affects whether the bees from that colony preferentially direct allogrooming at infected nestmates (Carr et al., 2020). We did not measure the aggression level of the colonies used in this study, which could explain why we saw such different effects across our cohort.

Figure 5.1

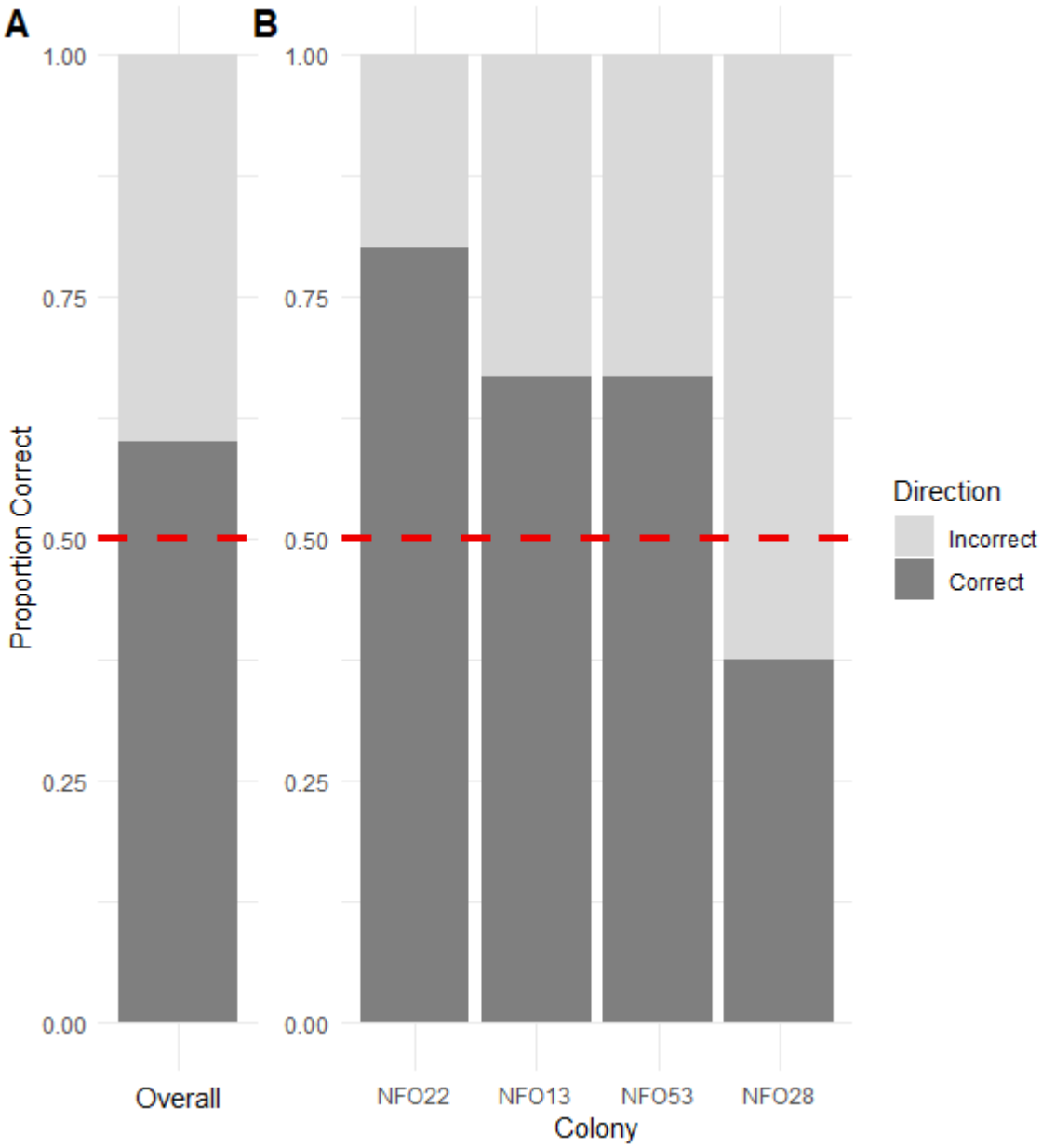



Figure 5.1: Honey bee foragers in our experiment groomed in the desired direction (an uninfected bee grooming its infected cagemate, "Correct") 50% more frequently than the reverse direction ("Incorrect"). A) Barplot of the proportion of correct and incorrect grooming interactions with all colonies grouped. Dotted red line represents a 50% chance threshold. B) The same data as shown in "A," but separated by colony.

### 5.3.1 Fat body gene expression

We measured the expression of five key immune genes in the immune-and-endocrine organ called the "fat body" and assessed whether infection status (infected/uninfected) and/or allogrooming behavior (groomer/recipient/non-behaving) affected these expression patterns. All analyses were performed as linear mixed models on normalized, log-transformed expression of the target gene. We included the behavioral category with or without infection status as a fixed effect and colony ID as a random effect. We used Akaike's Information Criterion (AIC) to determine the best model for each gene. We performed Dunnett's post-hoc test on final models that returned a significant or trend result to assess pairwise comparisons of interest within the treatment groups.

Only the model for *defensin-1* performed better when infection status was included along with behavior, and this gene was not significantly affected by either factor (Figure 5.2; log-transformed defensin-1: ANOVA: Infection Status: Wald $X^2_1$=1.2, p= 0.28; Behavior: Wald $X^2_2$=1.9, p= 0.38). We note that there is an increase in expression of *defensin-1* for infected controls relative to uninfected controls (i.e. the effect of infection alone), and although nonsignificant, it is similar in magnitude to results seen in previous research (Carr et al., 2020). Therefore, our sample size may not have been adequate in this study to statistically detect this subtle effect.

Figure 5.2

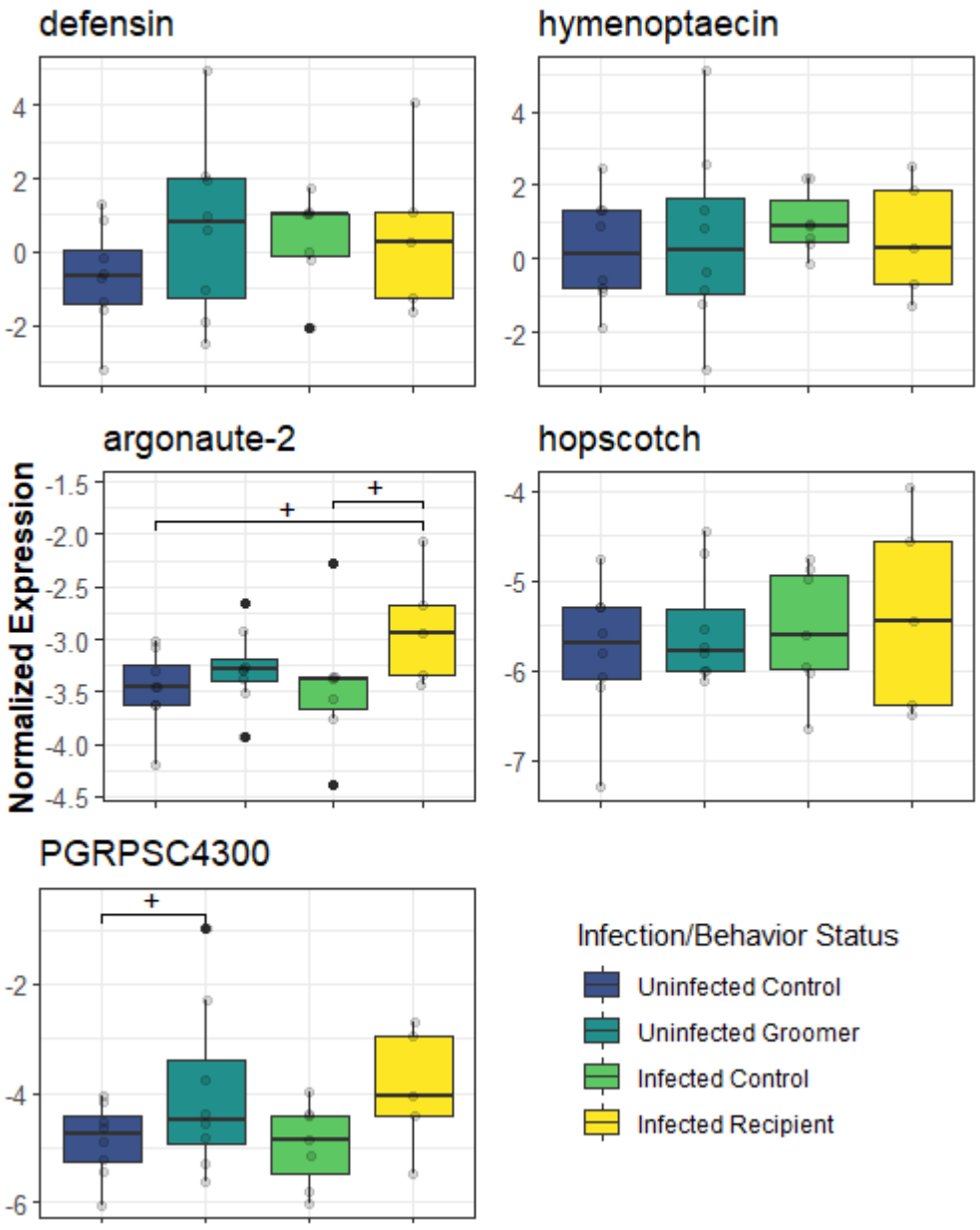



Figure 5.2: Allogrooming behavior affects the expression of some honey bee immune genes in the fat body. Boxplots of the normalized, log-transformed expression of: A) *defensin-1*; B) *hymenoptaecin*; C) *argonaute-2*; D) *hopscotch*; and E) *PGRPSC4300,* each split by infection status (Uninfected/Infected) and allogrooming behavior (Control/Groomer/Recipient). Linear mixed models for *argonaute-2* and *PGRPSC4300* showed a trend towards allogrooming behavior affecting expression of these genes. "+" represents $p < 0.1$, "*" represents $p < 0.05$ as assessed by a Dunnett post-hoc test.

All other genes measured in the fat body returned models that performed better without the inclusion of infection status (i.e. only considering allogrooming behavior). We found that allogrooming behavior did not affect the expression of either *hymenoptaecin* or *hopscotch* (Figure 5.2; log-transformed *hymenoptaecin*: ANOVA: Wald $X^2_2$=0.15, p= 0.93; log-transformed *hopscotch*: ANOVA: Wald $X^2_2$=1.26, p= 0.53).

When we examined the expression of *argonaute-2*, we found a trend towards higher expression in the recipients of allogrooming relative to both infected and uninfected controls (Figure 5.2; log-transformed *argonaute-2*: ANOVA: Wald $X^2_2$=5.4, p= 0.066; Dunnett's test: uninfected groomer-uninfected control: p=0.63, infected recipient-uninfected control: p=0.057, infected control-uninfected control: p=0.99; infected recipient-infected control: p=0.10). This result suggests that an infected individual who receives allogrooming experiences heightened expression of this important antiviral defense effector molecule when she is allogroomed by a nestmate, even above the level that is induced by infection alone.

Additionally, when we examined *PGRPSC4300*, we found a trend towards higher expression of this gene in groomers relative to uninfected controls (Figure 5.2; log-transformed *PGRPSC4300*: ANOVA: Wald $X^2_2$=5.4, p= 0.067; Dunnett's test: uninfected groomer-uninfected control: p=0.10, infected recipient-uninfected control: p=0.17, infected control-uninfected control: p=0.99, infected recipient-infected control: p=0.20). This result suggests that worker bees who undertake the risky behavior of grooming an infected nestmate increase their expression of this important pathogen recognition protein, possibly enhancing their bodies' ability to detect and quickly neutralize any infectious particles they pick up during the interaction.

### 5.3.2 Brain gene expression

We measured the expression of two genes that have been implicated in social responsiveness in the brain and assessed whether infection status and/or allogrooming behavior affected their expression. All analyses were performed as linear mixed models on endogenous-control-corrected expression of the gene-of-interest with the behavioral type and infection status as a fixed effect and colony ID as a random effect. The models for both *ftz-f1* and *Nup98-96* returned similar results whether infection status was included with behavior or not, so we are choosing here to report the results from the models that included it. We performed Dunnett's post-hoc test on final models that returned a significant result to assess pairwise comparisons of interest within the treatment groups.

We found that expression of both *ftz-f1* and *Nup98-96* was associated with allogrooming behavior (Figure 5.3). Groomers showed significantly higher expression of *ftz-f1* than controls, and recipients showed a trend towards higher expression of this gene as well (*ftz-f1*: ANOVA: Wald $X^2_3$=10.1, p= 0.02; Dunnett's test: uninfected groomer-uninfected control: p=0.02, infected recipient-uninfected control: p=0.09, infected control-uninfected control: p=0.66, infected recipient-infected control: p=0.23). For *Nup98-96*, only groomers showed significantly higher expression than controls (*Nup98-96*: ANOVA: Wald $X^2_3$=7.7, p= 0.05; Dunnett's test: uninfected groomer-uninfected control: p=0.046, infected recipient-uninfected control: p=0.20, infected control-uninfected control: p=0.77, infected recipient-infected control: p=0.38). These results taken together suggest that a bee that performs allogrooming experiences changes to diverse genes within her brain that may promote further social responsiveness. Interestingly, bees that receive allogrooming seem

to show this effect as well (albeit more moderately), which could drive a positive feedback loop of social cohesion within the colony.

Figure 5.3

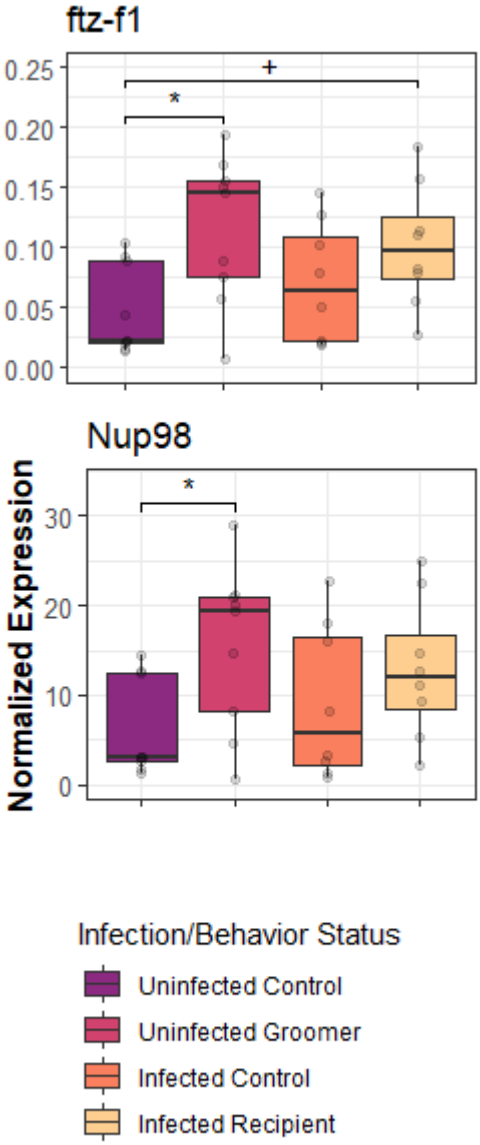


Figure 5.3: Allogrooming behavior affects the expression of genes implicated in social responsiveness in the brain. Boxplots of the normalized expression of: A) *ftz-f1*; and B) *Nup98-96*, split by infection status (Uninfected/Infected) and allogrooming behavior (Control/Groomer/Recipient). Linear mixed models for both *ftz-f1* and *Nup98-96* showed statistically significant differences for allogrooming behavior and infection status. "+" represents $p < 0.1$, "*" represents $p < 0.05$ as assessed by a Dunnett post-hoc test.

## 5.4 Discussion

We found that allogrooming, a prosocial and social immunity-related behavior, is associated with changes in expression of both immune genes in immune tissue and socially responsive genes in brain tissue. Worker honey bees who performed allogrooming towards a nestmate experiencing an immune challenge showed changes in expression of multiple genes. First, they showed a trend towards heightened expression of *PGRPSC4300*, a gene that encodes a peptidoglycan recognition protein. These molecules recognize components of the bacterial cell wall and activate downstream components of the immune system to respond to the infection. Groomers additionally showed significantly higher expression of two different genes that have been implicated in social responsiveness in the brain, *ftz-f1* and *Nup98-96*. A previous study found these genes to be significantly differently expressed in bees that were more likely to respond to a social challenge (an aggressive intruder bee) as well as a social opportunity (a larva to nurse) than less-socially-responsive individuals (Shpigler, Saul, Corona, et al., 2017). We also found subtle differences in gene expression between bees who were experiencing an immune challenge vs. immune-stimulated bees who were not groomed. First, we found a trend towards increased expression of *argonaute-2*. *Argonaute-2* is a key component of honey bee antiviral defense as a part of the RNA-interference pathway. We additionally found a trend towards increased expression of *ftz-f1* in the recipients of allogrooming, similar to what we saw in groomers, suggesting that

receiving allogrooming might reinforce or increase social responsiveness in these individuals.

Three of the immune genes we analyzed in this study did not show changes in expression based on allogrooming behavior, including genes encoding the important antimicrobial peptides Defensin-1 and Hymenoptaecin as well as the gene *hopscotch* (an intermediate in the Jak/STAT immune pathway). The expression of these genes may be mediated more directly by other internal or external factors (S. W. Cole, 2013; Gibson, 2008; Jaenisch & Bird, 2003). Alternatively, given that we saw an effect on the expression of *PGRPSC4300* (which is upstream of the antimicrobial peptides), it is possible that allogrooming mediates these factors indirectly through pathogen recognition. This system would be particularly efficient, given that the production of these effectors would only be activated if the grooming interaction did indeed lead to an infection. The increase in *PGRPSC4300* expression in groomers is in concordance with the findings of Cini et al., who found that honey bees who allogroom more frequently perform better on bacterial clearance tests in the hemolymph (Cini et al., 2020). PGRPs are peptidoglycan recognition proteins, which recognize components of the bacterial cell wall and induce an immune response. Having more of these proteins could have caused the high-grooming individuals in that study to detect and clear bacterial infections before their numbers were able to multiply out of control.

That *argonaute-2* expression (a component of antiviral defense) in particular was upregulated in response to receiving allogrooming may in part be related to a particular honey bee parasite. One component of allogrooming in honey bees is that the groomer will bite at and attempt to remove parasitic *Varroa destructor* mites she finds on the body

surface of the recipient (Pritchard, 2016). This behavior is particularly important because Varroa mites are one of the most insidious honey bee pests and sometimes attach in places that bees are unable to access with self-grooming (Božič & Valentinčič, 1995; Noël et al., 2020; Pritchard, 2016). Varroa mites transmit many viruses, including the devastating deformed wing virus (DWV) with a high frequency and heightened pathogenicity compared to bee-to-bee transmission (de Miranda & Genersch, 2010). Given these links between allogrooming and Varroa mites, and Varroa mites and viral pathogens, the activation of *argonaute-2* in recipients of allogrooming may be particularly protective of this effect. Honey bee workers that are self-grooming to remove *Varroa* mites have also shown changes in gene expression as a result of this behavior, although *argonaute-2* was not measured in that study (Hamiduzzaman et al., 2017).

Both of the social responsiveness genes we measured were affected by allogrooming. These genes were initially identified in a broad-scale RNA-seq study that found that they were differentially expressed in individuals who showed a high degree of social responsiveness across contexts (Shpigler, Saul, Corona, et al., 2017). The social contexts in that study were a social challenge (where a bee was given an opportunity to aggressively defend her group against an intruder) and a social opportunity (where a bee was given an opportunity to tend to a larva). Our work extends the range of social contexts in which these genes are implicated—to the affiliative adult interaction of allogrooming. We additionally found evidence that there could be a mutual reinforcement of social cohesion through the activation of these genes. In the case of *ftz-f1*, both groomers and recipients showed altered expression. Social responsiveness could therefore spread through a colony as one bee allogrooms another, causing the recipient to become more socially

responsive across contexts and potentially causing other individuals she interacts with to become more socially responsive as well. Future studies could explicitly test whether such a positive feedback loop exists in this system.

The findings of our study plus the social responsiveness study by Shpigler et al. could help explain a puzzling finding in bees—aggressive individuals and colonies tend to be healthier and more robust by a variety of metrics, whereas in vertebrates, aggression is more often associated with poor health outcomes (Border et al., 2019; Carr et al., 2020; Heinzeller et al., 1988; Lemonnier et al., 2022; Martin & Medina, 2004; Quque et al., 2022; Rittschof et al., 2015; Rittschof et al., 2019; Wray et al., 2011). Our and Shpigler et al.'s work instead point to aggression in honey bees serving as a sign of high social cohesiveness rather than only a health-busting stressor (Shpigler, Saul, Corona, et al., 2017). Worker bees from aggressive colonies allogroom more frequently and preferentially direct that allogrooming specifically towards sick nestmates (Carr et al., 2020). Our study has found that allogrooming is associated with changes in the expression of key immune genes that may promote positive health outcomes for both the groomers and recipients, which would occur more frequently in high-aggression colonies. This result would explain why aggressive bees and colonies tend to be healthier when it comes to disease-related metrics. But these allogrooming interactions are also associated with changes in the expression of social responsiveness genes first identified by Shpigler et al. The activation of these genes would then go on to heighten aggressive responsiveness while further strengthening the colony in other areas such as enhanced responsiveness to the demands of the brood. This reinforcement cycle would lead to colonies being more robust in multiple dimensions (immunity, brood production, nest defense). Indeed, a lack of this social cohesion (i.e.

social dysfunction) has been proposed to be part of the suite of stressors (along with overt disease) that lead to colony collapse disorder (CCD) in honey bees (Kaplan, 2012).

When it comes to the expression of the socially responsive genes in groomers, our study cannot definitively weigh on whether allogrooming independently causes the expression of these genes to increase or whether bees that naturally show higher expression of these genes are more likely to be the ones who allogroomed first (and were therefore chosen to serve as the behaving group rather than the non-behaving control). We have two reasons to believe that the former situation is more likely, however. First, one of the two genes (*ftz-f1*) was additionally elevated in recipients of grooming. This effect was almost certainly caused by the immediate action of allogrooming, since the recipients did not initiate the interaction (and we never observed a "grooming invitation dance" by the recipients in our study, (Land & Seeley, 2004)). Second, though we chose the first pair that allogroomed in each round to use as our behaving pair and forced the second pair in that round to act as a non-grooming control, allogrooming was common and occurred quickly in our study. We have no reason to suspect that the pairs that ended up as controls would have been less likely to groom at some point in the 15-minute observation window than the pairs that happened to groom first, had they been given the opportunity to do so. We additionally saw no relationship between the latency to allogroom and the expression levels of these genes (see Appendix 4, Fig. A4S1). That finding might have suggested that bees with higher endogenous expression of these genes were faster to groom and therefore more likely to end up in the behaving treatment rather than the control, but the lack of relationship suggests that this was not the case.

The combination of direct and indirect effects of allogrooming can make it difficult to interpret the adaptive function of social immunity phenotypes. Our study unites the social and immune functions of allogrooming by showing that they are subject to manipulation by a common mechanism: gene expression. Allogrooming offers direct benefits to the recipients by enhancing the expression of a gene relevant to antiviral defense in addition to other known benefits, such as pathogen and parasite removal from the body surface. The cost of disease risk to the groomer may be partially offset by the enhanced expression of genes related to pathogen recognition. And finally, allogrooming seems to provide a source of social reinforcement for both the groomer and recipient by elevating the expression of genes implicated in social responsiveness. These findings are in agreement with literature in vertebrates that demonstrates that social touch and prosocial behavior enhance both immune function and social bonds (or, in our subjects, social cohesion) (Ang et al., 2012; Cohen et al., 2015; Nelson-Coffey et al., 2017; Spruijt et al., 1992). Our work demonstrates that this phenomenon extends to invertebrates as well and provides a clear view of how enmeshed the brain, behavior, and the immune system are in highly social organisms.

# CONCLUDING REMARKS

This research aimed to further our understanding of how early life experiences and social interactions can alter behavior and health in the western honey bee (*Apis mellifera*). Overall, we identified diverse processes linking early-life experiences, social behavior, aggression, sensory detection of social cues, and health in these insects. We began by compiling the current state of knowledge on how early life experiences affect adult behavior in insects. We also argued for expanding the use of insects to understand how developmental behavioral plasticity leads to lasting change in the brain and behavior. This literature review has already contributed to this field of study, as it has been cited in multiple works since it was published.

Our first aim was to establish whether a key determinant of health, early-life nutrition, showed naturally occurring variation in honey bees. We found a striking degree of among-colony variation in the total dry weight, proteins, lipids, and carbohydrates in samples of worker jelly. Curiously, this variation was not well explained by any of the social- and health-related factors we examined, including colony-level aggression, site of the colony, or colony genetic strain. This chapter establishes that there is ample variation for selection to act on in the context of developmental plasticity. Future work could establish whether this variation leads to different outcomes in adulthood for the insects raised in these conditions.

Our second aim was to assess whether nursing behavior is altered by social pheromones and the colony-level aggressive social context. We found that this key social interaction between adult workers and larvae is affected by complex dynamics of social signals and social context; nurses from high-aggression colonies changed larval care

behaviors in the presence of the honey bee alarm pheromone, despite not being specialized for the task that alarm pheromone is typically associated with (nest defense). Meanwhile, nurses from low-aggression colonies did not show this pattern. This chapter establishes that social cues can cross the boundaries of the behaviors they typically regulate and alter performance on other tasks, even for individuals who are not particularly responsive to these cues in their normal context. These results additionally illuminate that colony-level social aggression phenotypes may not be limited to colony defense; instead, these social dynamics may instead represent a broad shift in overall colony priorities that affects multiple aspects of colony function. Finally, this work provides a potential mechanistic explanation for the previously established developmental behavioral plasticity seen with honey bee aggression: that differences in the frequency and/or timing of larval care in high- versus low-aggression colonies leads to different behavioral outcomes in adulthood. (Rittschof et al., 2015). Future work could explicitly test larval outcomes when raised under these different, naturally occurring care regimes.

Our third aim was to establish whether differences in task specialization and colony-level aggressive social context are associated with differences in peripheral sensitivity to stimuli that coordinate those tasks. We found limited support for this idea. Foragers showed overall slightly higher antennal sensitivity to the honey bee alarm pheromone than nurses, as expected based on their behavioral sensitivity to this pheromone. On the other hand, we did not find this pattern for a larval begging signal. Instead, sensitivity to this pheromone was more strongly associated with colony-level aggressive social context. At relatively high (but behaviorally relevant based on our second aim) concentrations, we found that nurses from low-aggression colonies had lower

antennal sensitivity to the larval begging signal than nurses from high-aggression colonies. This pattern reversed at lower concentrations of the begging signal. Meanwhile, foragers from high- and low-aggression colonies showed no difference in antennal sensitivity at the same behaviorally relevant concentration of the begging signal. This result reveals that individual behavioral specialization is not proximally driven by which individuals are detecting the relevant stimuli most strongly. Instead, there are likely complex interactions between peripheral sensitivity, higher-order brain processing of these signals, and internally-guided motivations that shape task preferences in these insects. Future studies could seek to determine what factors are working in concert with peripheral sensitivity to drive individual motivation for particular tasks. This chapter furthermore adds another dimension to our understanding of the potential mechanisms by which developmental behavioral plasticity of aggression occurs in honey bees; in addition to the effects on the timing of nurse visitation we discovered in Aim 2, here we see that nurses from high-aggression colonies may be less sensitive to low levels of larval hunger but more sensitive when larval begging cues increase drastically. If the nurses in high-aggression colonies are allowing for low (i.e. non-lethal) levels of starvation of the larvae, and larvae raised in high-aggression colonies are more aggressive as adults, these results together could explain some of the physiological similarities between aggressive adults and starvation-resilient adults (Pearce et al., 2001; Rittschof et al., 2015; Robinson, 1987a, 1987b; Wang, Campbell, et al., 2016; Wang, Kaftanoglu, et al., 2016). Futures studies could explicitly test these potential links between metabolism, starvation, and aggression in the context of developmental plasticity.

Lastly, our fourth aim was to explore how an acute allogrooming event affects social dynamics and health. Social and immune benefits of allogrooming and other forms of social touch have been well established in vertebrates (Dunbar, 2010; Hart & Hart, 2018). The relationship between immune function and behavior in invertebrates has received much less attention, though its importance is beginning to be recognized (Eleftherianos et al., 2023). This chapter brings an exciting new contribution to this burgeoning field. We examined social and immune effects in both individuals that received allogrooming and, importantly, individuals who performed it. We found that groomers and recipients showed altered expression of both immune genes and genes that have been implicated in broad social responsiveness in honey bees. We expect that the immune gene expression changes we observed in recipients of allogrooming would be beneficial in helping fight off any infection they are currently experiencing. The gene expression changes observed in groomers may be beneficial in proactively protecting them from catching an infection from the individual they are grooming. Similarly, we expect that the changes in expression of social responsiveness genes would promote social responsiveness in both the groomer and the recipient, enhancing social cohesion within the colony. This would likely be a beneficial change, as social dysfunction in honey bee colonies is known to be harmful (Kaplan, 2012). Future work could explicitly test these assumptions to verify that the social and immune changes we observed are indeed advantageous. Overall, the findings of this chapter mirror what has been found in vertebrates, where allogrooming interactions are associated with improvements in both health and social outcomes (Henazi & Barrett, 1999; McFarland). Finding these patterns in invertebrates suggests that the entanglement of social and immune benefits may be deeply fundamental to social evolution

and group living. Furthermore, we have established with this work that changes in gene expression act as a common mechanism on both of these facets of the socio-immune dynamic.

This work benefits from a robust diversity of approaches. We used behavioral observations, neurophysiology, transcriptomics, and techniques to study nutrition. Our approach to interpreting our results additionally considers implications across different biological and time scales. The research in this dissertation does have certain limitations. For example, though the second chapter achieved our aim to characterize the degree of variation in larval nutrition, we failed to determine what factors are associated with this variation. The fourth chapter provides new insights into the association between observed task specialization and peripheral sensory sensitivity, but this work would benefit from future additions that explicitly and temporally tie this sensitivity to behavior or additions that consider a broader range of odorants. And finally, the fifth chapter discovers exciting patterns relating allogrooming to immune function and social responsiveness, but our sample size was small enough that caution must be used in stating the strength of these results. Hopefully future experiments will reinforce our findings from this chapter and answer the intriguing new questions raised more generally in this dissertation.

Overall, this work contributes to the study of how previous experiences affect behavior and health. We chose to study these phenomena in honey bees. As developmental and behavioral plasticity are common across animals, though, the implications of our work are not limited to these insects. We have shown clearly and consistently throughout this work that social context is important for interpreting behavioral results. We primarily demonstrated this fact with colony-level aggressive social context, but this phenomenon is

likely true for other social contexts as well. We have provided examples of multiple experiences that can contribute to developmental and behavioral plasticity in honey bees: nutrition, parental care, and adult social interactions. We have also linked these experiences to multiple physiological mechanisms that can affect behavioral and health outcomes: sensory sensitivity and gene expression. Our work provides explanations for previous research gaps and puzzling questions in honey bee biology. For example, we have identified possible explanations for how the aggressive social environment "gets under the skin" of larvae to cause lasting behavioral change (Rittschof et al., 2015), why the physiology of aggressive adults mirrors that of starvation-resilient adults (Wang, Campbell, et al., 2016; Wang, Kaftanoglu, et al., 2016), and why aggressive individuals and colonies tend to be healthier than their less-aggressive counterparts (Rittschof et al., 2015; Rittschof et al., 2019). This work additionally points to future practical avenues to promote health and vitality of honey bee colonies. Previous work has focused largely on factors like treating diseases, providing adequate food sources, and minimizing pesticide exposure (Pettis & Delaplane, 2010). These approaches are certainly warranted. But given the persistent difficulties in reversing declines of these important pollinators, new approaches are clearly needed (Maini et al., 2010). Our work provides key foundational knowledge on a less-well-studied component of honey bee health: how social factors can feed into larger patterns of health in these insects. In conclusion, previous experiences, social behavior, and health show strong ties that affect many aspects of the biology of these fascinating insects.

# APPENDICES

[APPENDIX 1. Supplemental material for Chapter 2]

Table A1S1: Table listing Colony ID, colony aggression level, genetic strain, site of the colony, and the date the worker jelly was collected.

| Colony ID | Aggression Level | Genetic Strain | Site | Date Collected |
|---|---|---|---|---|
| H1 ("NFUPPYY") | High | Italian | Alpha | 29 June 2019 |
| L1 ("NFU42") | Low | Russian Hybrid | Alpha | 29 June 2019 |
| H2 ("NFO13") | High | Italian | Beta | 30 June 2019 |
| L2 ("NFO6") | Low | Russian Hybrid | Beta | 30 June 2019 |
| H3 ("NFO38") | High | Italian | Beta | 30 June 2019 |
| H4 ("NFL25") | High | OTHER | Alpha | 5 July 2019 |
| L3 ("NFL27") | Low | OTHER | Alpha | 5 July 2019 |
| H5 ("NFO64") | High | Italian | Beta | 6 July 2019 |
| L4 ("NFO12") | Low | Russian Hybrid | Beta | 6 July 2019 |
| L5 ("NFO34") | Low | Russian Hybrid | Beta | 6 July 2019 |
| H6 ("NFU11") | High | Russian Hybrid | Alpha | 11 July 2019 |
| L6 ("NFU2") | Low | Russian Hybrid | Alpha | 11 July 2019 |
| L7 ("D1") | Low | Wild Stock | Gamma | 19 July 2019 |

| | | | | |
|---|---|---|---|---|
| H7 (“D2”) | High | Wild Stock | Gamma | 19 July 2019 |
| H8 (“D3”) | High | Wild Stock | Gamma | 20 July 2019 |
| L8 (“D4”) | Low | Wild Stock | Gamma | 20 July 2019 |
| H9 (“D5”) | High | Wild Stock | Gamma | 21 July 2019 |
| L9 (“D6”) | Low | Wild Stock | Gamma | 21 July 2019 |

Fig. A1S1

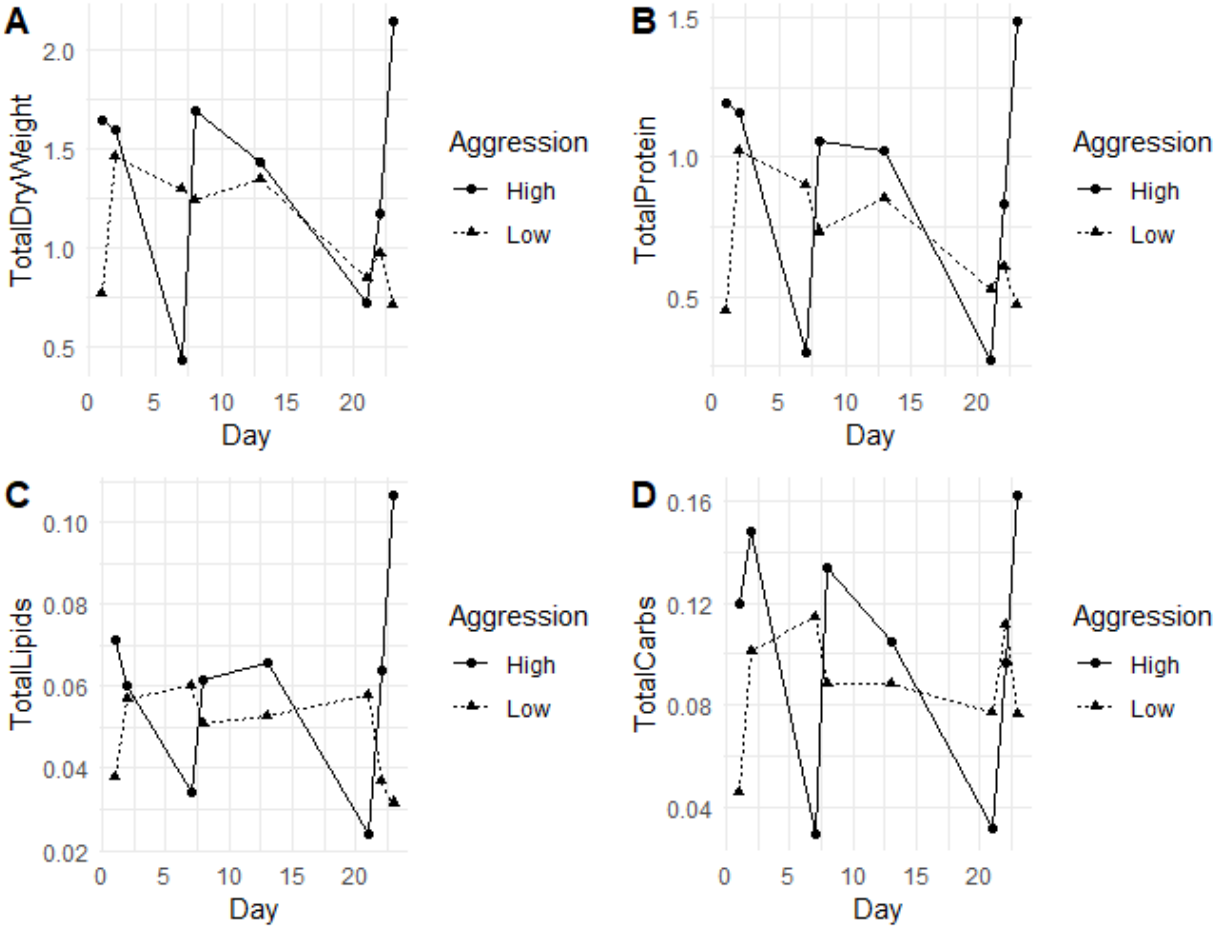


Figure A1S1: There is no clear seasonal trend over the course of the 23 days that samples were collected. All charts show the average mass in mg per nutrient by experimental day (with the first collection day being Day 1), separated by colony aggression level. A) Total dry weight, B) total proteins, C) total lipids, D) total carbohydrates.

Figure A1S2

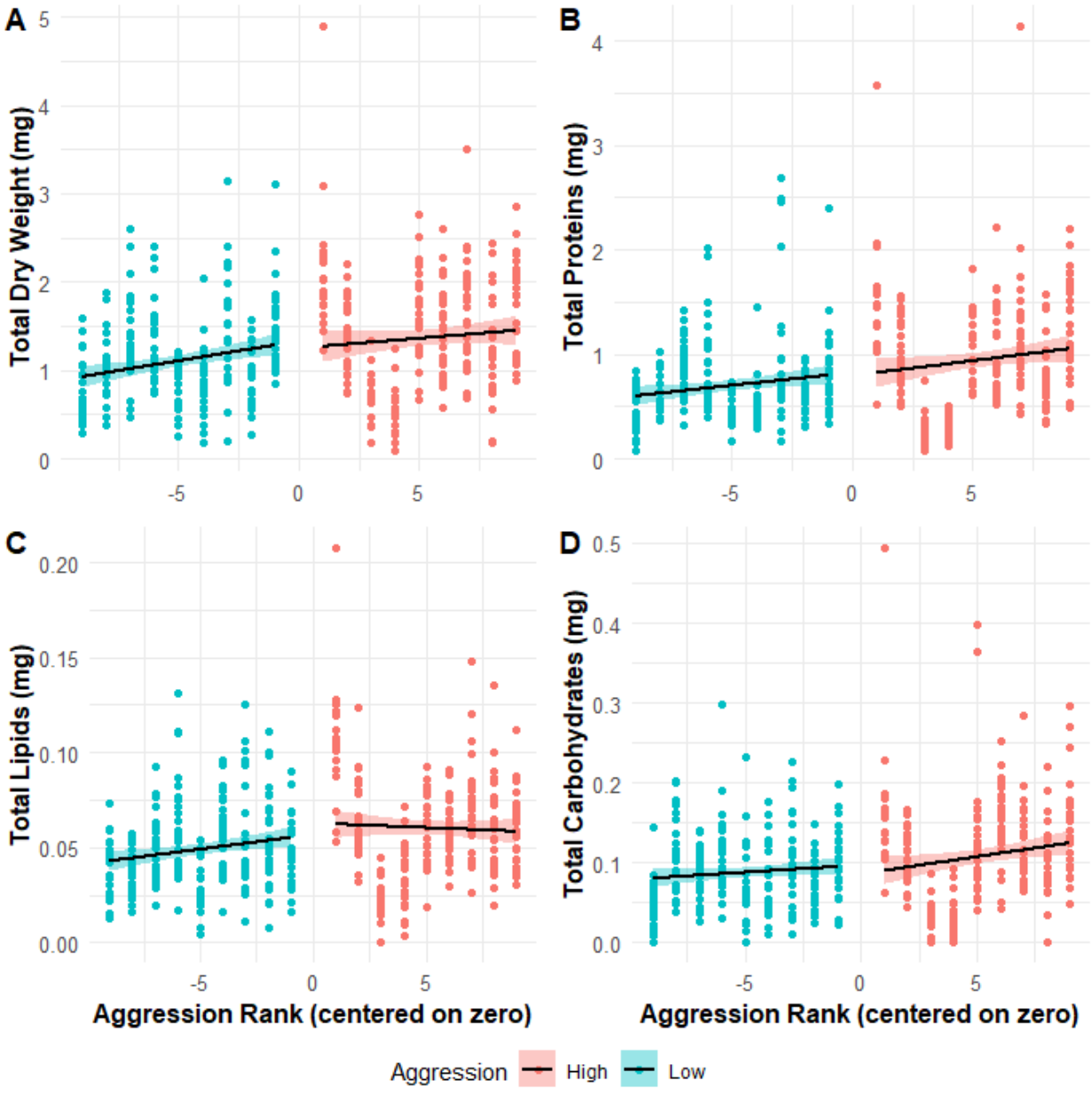


Figure A1S2: Ranked aggression score is not predictive of worker jelly nutritional profiles. Scatterplots of aggression ranks (centered around zero) versus A) total dry weight, B) total proteins, C) total lipids, and D) total carbohydrates of honey bee worker jelly samples. Colors are indicative of how colonies were grouped into the high- versus low-aggression categories in the previous analysis where aggression was treated as a binomial variable.

Fig. A1S3

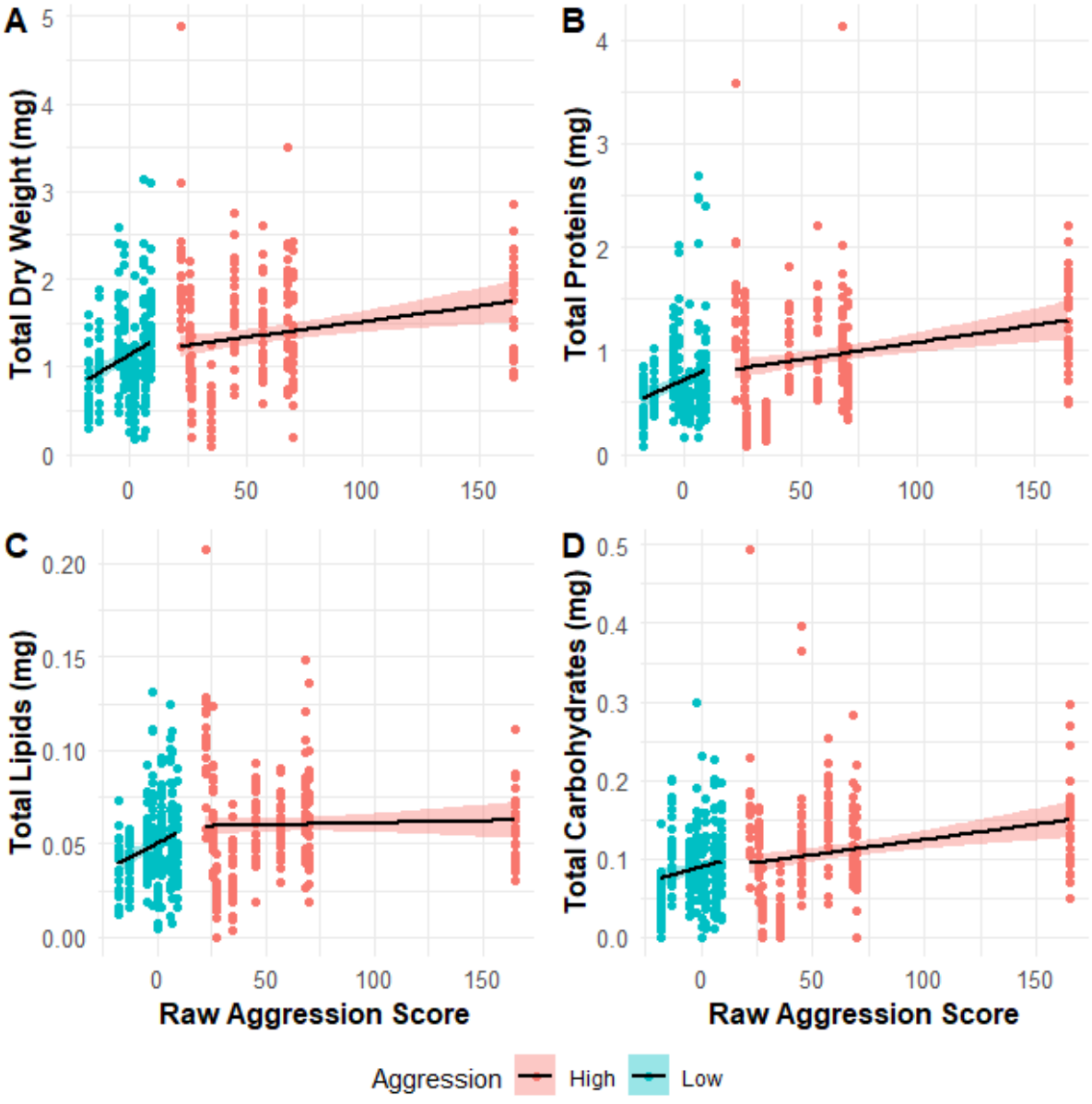


Figure A1S3: Continuous aggression score is not predictive of any of the nutrients we measured. Scatterplots of raw aggression score versus A) total dry weight, B) total protein, C) total lipids, and D) total carbohydrates of honey bee worker jelly samples. Colors are indicative of how colonies were grouped into high- versus low-aggression colonies in the previous analysis.

Fig. A1S4

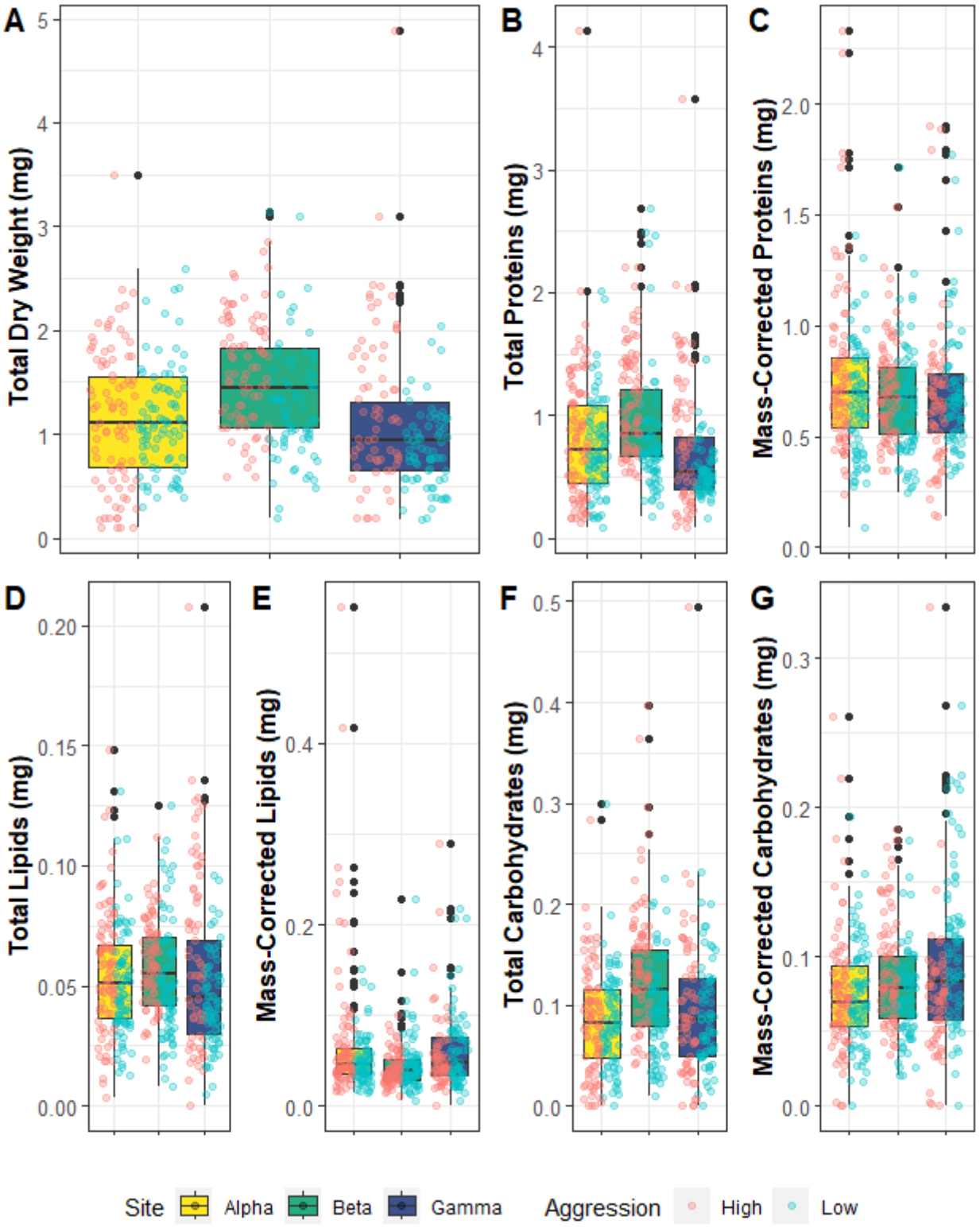

A
Total Dry Weight (mg)
0
1
2
3
4
5
B
Total Proteins (mg)
0
1
2
3
4
C
Mass-Corrected Proteins (mg)
0.0
0.5
1.0
1.5
2.0
D
Total Lipids (mg)
0.00
0.05
0.10
0.15
0.20
E
Mass-Corrected Lipids (mg)
0.0
0.2
0.4
F
Total Carbohydrates (mg)
0.0
0.1
0.2
0.3
0.4
0.5
G
Mass-Corrected Carbohydrates (mg)
0.0
0.1
0.2
0.3
Site
Alpha
Beta
Gamma
Aggression
High
Low

Figure A1S4: Site was not predictive of worker jelly nutritional content. Samples of worker jelly were collected from colonies in three yards: “Alpha” and “Beta” were approximately 1 mile apart, while “Gamma” was approximately 9 miles away from the other two sites. Linear mixed models of each nutrient with site as a fixed effect and colony ID as a random effect all showed no significant differences. Boxplots of A) total dry weight , B) total proteins, C) mass-corrected proteins, D) total lipids, E) mass-corrected lipids, F) total carbohydrates, and G) mass-corrected carbohydrates of worker jelly samples at these three sites. Each dot represents one sample, and the color of the dots represents the aggression level of the colony they were sampled from.

Fig. A1S5

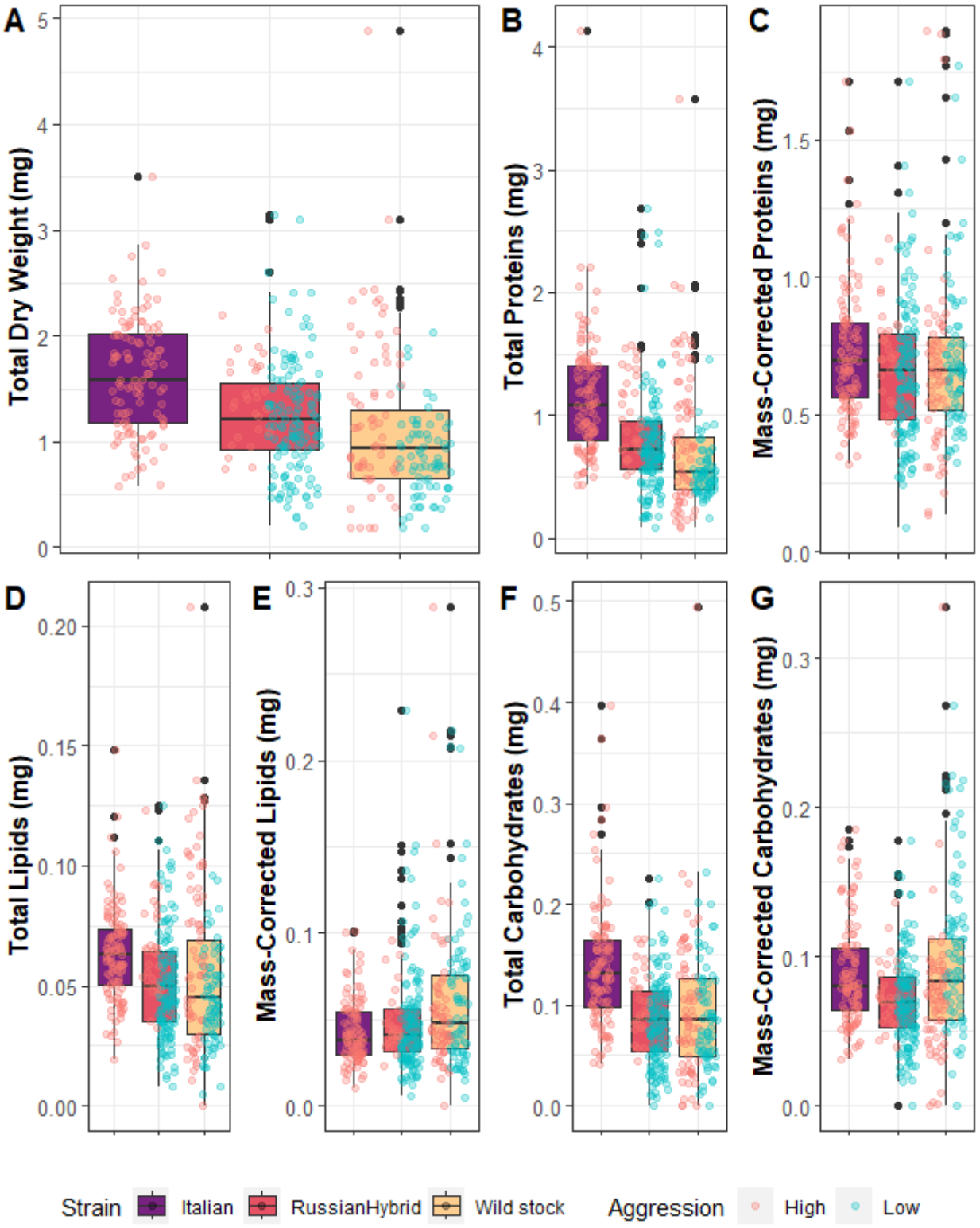

A
Total Dry Weight (mg)
B
Total Proteins (mg)
C
Mass-Corrected Proteins (mg)
D
Total Lipids (mg)
E
Mass-Corrected Lipids (mg)
F
Total Carbohydrates (mg)
G
Mass-Corrected Carbohydrates (mg)
Strain
Italian
RussianHybrid
Wild stock
Aggression
High
Low

Figure A1S5: Genetic strain of the colony was not predictive of worker jelly nutritional content. Some colonies were established as packages with strains advertised as “Italian” (4 colonies) and “Russian Hybrid” (6 colonies) at the beginning of the season. All but one of the package colonies still contained the original foundress queen, the other being a direct descendant of the foundress. Other colonies were of mixed local genetic stock (“Wild Stock,” 6 colonies). Linear mixed models of each nutrient with strain as a fixed effect and colony ID as a random effect all showed no significant differences. Boxplots of A) total dry weight, B) total proteins, C) mass-corrected proteins, D) total lipids, E) mass-corrected lipids, F) total carbohydrates, and G) mass-corrected carbohydrates in the worker jelly from colonies of these three genetic strains. Each dot represents one sample, and the color of the dots represents the aggression level of the colony they were sampled from.

[APPENDIX 2. Supplemental material for Chapter 3]

Experimental Setup

Figure A2S1

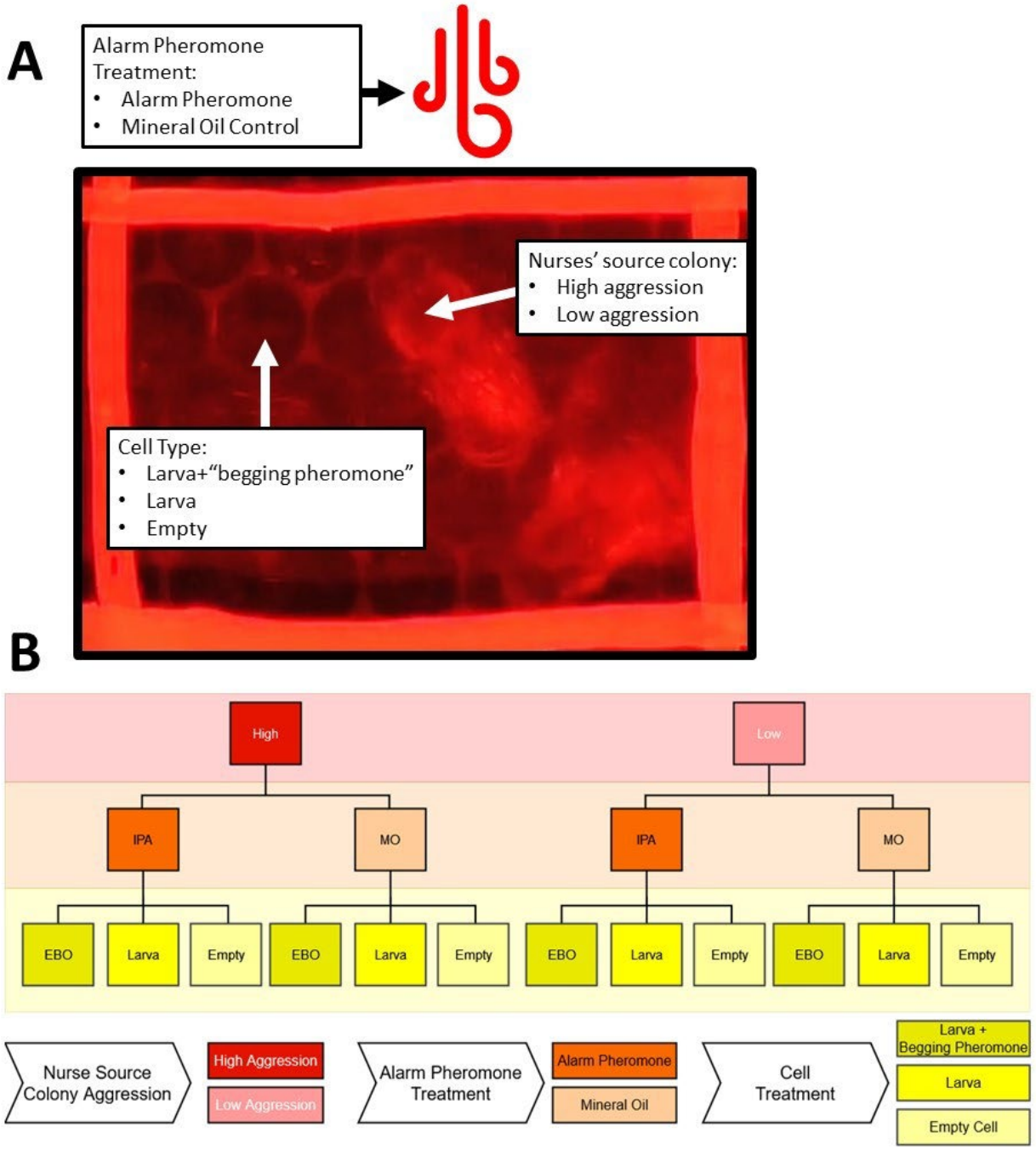


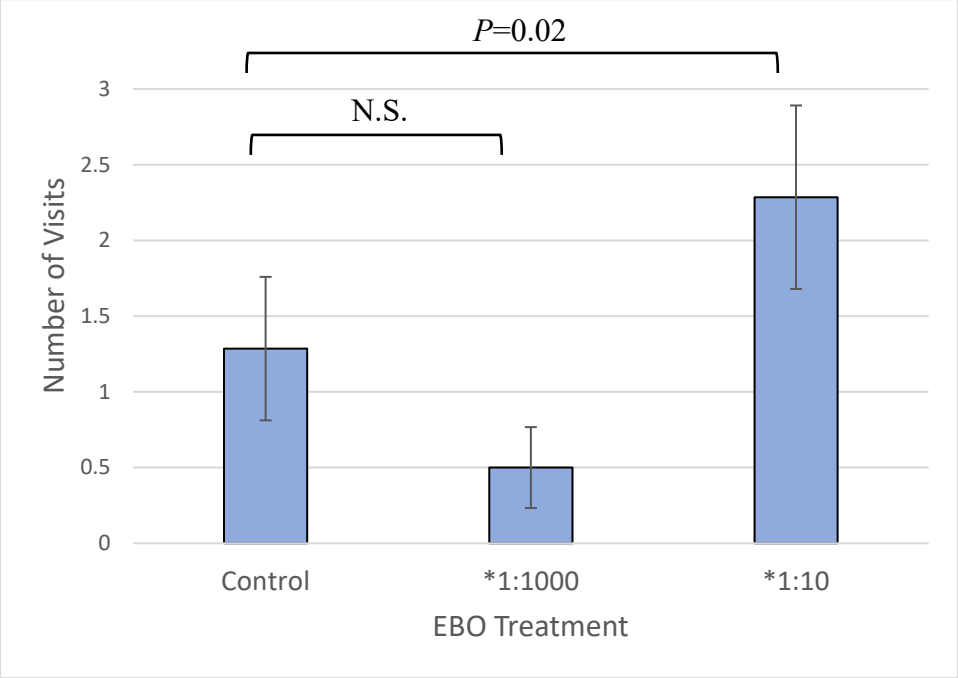
Figure A2S1: Diagram showing the experimental setup for our experiment. A) Three different variables were considered in this study. We examined the effects of the nurse source colony aggression (high/low), the alarm pheromone treatment (IPA "alarm pheromone"/mineral oil control), and the cell type (larva with extra EBO "begging pheromone"/larva alone/empty cell control). B) All combinations of all treatments were considered in this study, in addition to replication at the level of the colony.

e-β-ocimene Pilot Study

We performed a small pilot study to test what concentration of e-β-ocimene (EBO) would elicit a response from our nurse bees. The methods were the same as used in the paper, except that we did not apply an alarm pheromone treatment. Briefly, we obtained a frame of brood and applied one of three EBO treatments to larval cells on the frame: 1) an untreated cell (control), 2) a cell to which we added 10uL of 1:1000 EBO in mineral oil (amount calculated modified from the findings of He et al. 2016 to fit our methods), and 3) a stronger stimulus of 10uL of 1:10 EBO (n=7 per treatment). We then drew nurse bees onto the frame, sourced from a colony that was not otherwise used in the experiment. We placed the frame into our observation hive under red light conditions and video recorded the frame for 30 minutes. We counted every time a nurse bee visited each marked cell (as described in "Methods").

In this pilot study, we found that only the 1:10 concentration increased visits (Fig. A2S2) (ANOVA, $F=4.79$, $p=0.02$; Tukey post-hoc comparisons: Control vs. 1:1000, $Q=2.19$, $p=0.29$; Control vs. 1:10, $Q=4.38$, $p=0.02$).

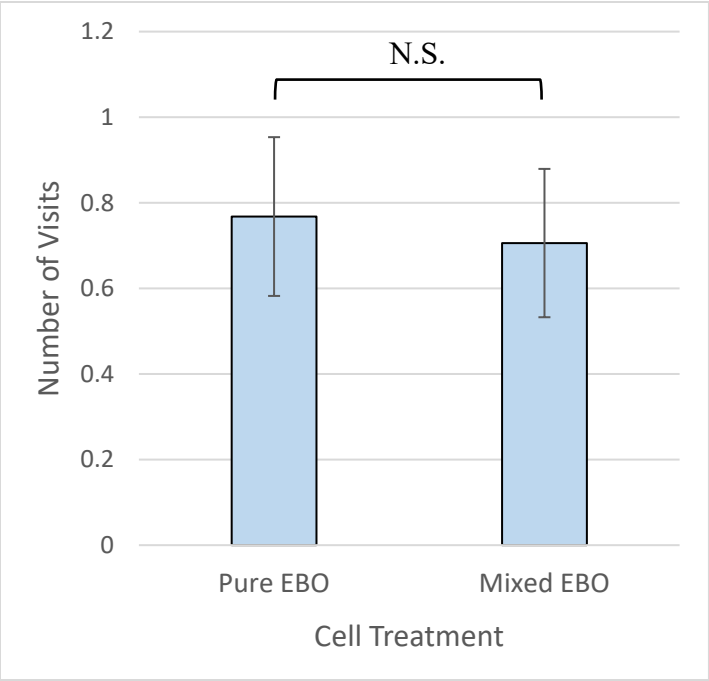
Figure A2S2




Pure versus Racemic e-β-ocimene Analysis

For the first four rounds of our experiment, we used both a pure form of EBO and a racemic mixture that had been used in previous experiments (as described in "Methods"). When these four rounds had been completed, we compared the number of nurse visits to cells with Pure EBO and racemic EBO to see if there was a difference in the response of the nurse bees to these two compounds (n=107 cells). We found that there was no difference between the groups that received pure EBO and those that had received the racemic mixture (Fig. A2S3) (Mann-Whitney U Test, z=0.19, p=0.85).

Figure A2S3




Models for number of nurse visits

Global model: Number of Nurse Visits = Nurse source colony aggression + IPA application + Cell type + Nurse source colony aggression*IPA application + Nurse source colony aggression*Cell type + IPA application*Cell type + Nurse source colony aggression*IPA application*Cell type + (1|Year) + (1|Colony ID) + (1|IPA Treatment Order)

Final model based on AICc: Number of Nurse Visits = Nurse source colony aggression + IPA application + Cell type + Nurse source colony aggression*IPA application + (1|Year) + (1|Colony ID) + (1|IPA Treatment Order)

Models for latency to the first visit

Global model: Latency = Nurse source colony aggression + IPA application + Cell type + Nurse source colony aggression*IPA application + Nurse source colony aggression*Cell type + IPA application*Cell type + Nurse source colony aggression*IPA application*Cell type + (1|Year) + (1|Colony ID) + (1|IPA Treatment Order)

Final model based on AICc: Latency = Nurse source colony aggression + IPA application + Nurse source colony aggression*IPA application + (1|Year) + (1|Colony ID) + (1|IPA Treatment Order).

Table A2S1

| Factor | Wald $X^2$ value (DF) | *P*-value |
|---|---|---|
| Nurse source colony aggression | 0.0003 (1) | *P*=0.98 |
| IPA application | 0.06 (1) | *P*=0.80 |
| Nurse source colony aggression*IPA application | 2.4 (1) | *P*=0.12 |

Figure A2S4

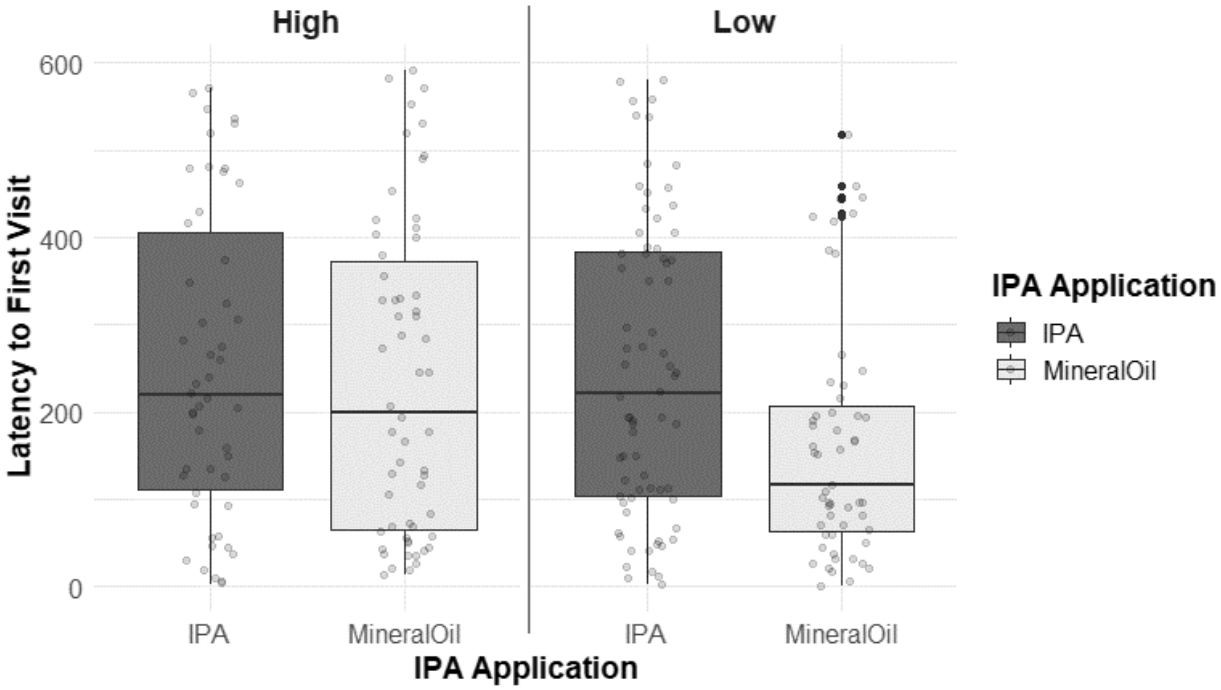



Figure A2S4. The latency to the first visit of each cell was not significantly affected by any of the explanatory variables in our final model. Boxplot of the latency (in seconds) to the first visit for each cell that received at least one visit. The final model included nurse source colony aggression (High vs. Low), alarm pheromone (IPA) application, and their interaction, but none of these effects was significant.

Table A2S2: Pairwise analysis of directionality of crosses per timepoint. Estimated marginal means comparison of the number of crosses up versus down at each timepoint for each combination of aggression and alarm pheromone (IPA) treatment. Values displayed have been treated with a Bonferroni correction for multiple comparisons.

| | High-aggression IPA | High-aggression Mineral oil | Low-aggression IPA | Low-aggression Mineral oil |
|---|---|---|---|---|
| -2 minutes | P=1 | P=0.67 | P=1 | P=1 |
| -1 minutes | P=1 | P=1 | P=1 | P=1 |
| 0 minutes | P=0.0096 | P=1 | P=1 | P=1 |

| | | | | |
|---|---|---|---|---|
| (exposure to stimulus) | | | | |
| 1 minute | P=1 | P=0.21 | P=1 | P=1 |
| 2 minutes | P=1 | P=1 | P=1 | 0.86 |
| 5 minutes | P=1 | P=1 | P=1 | P=1 |

[APPENDIX 3. Supplemental material for Chapter 4]

Fig. A3S1

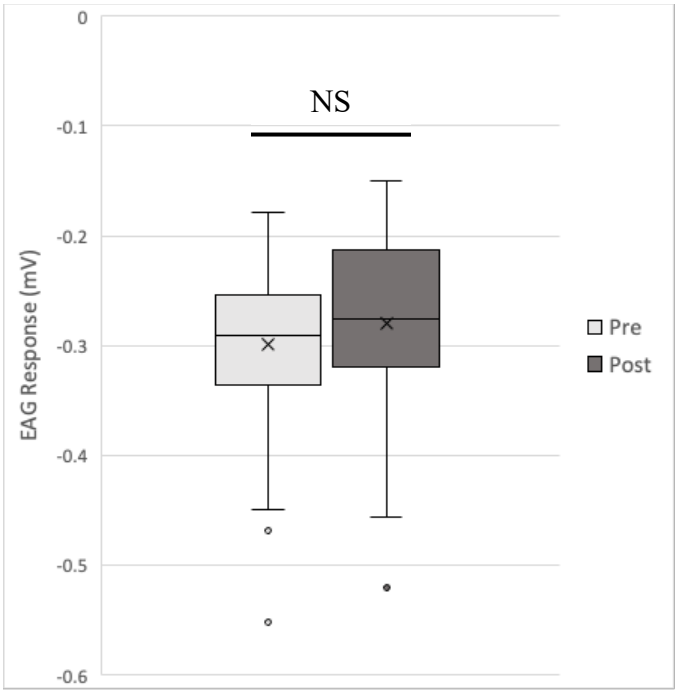



Figure A3S1: There is no significant difference between response to 1:10 IPA before (pre) and after (post) the experimental recordings, demonstrating that preparations did not significantly degrade over the course of the recording timeframe. Boxplot of pre- and post-experimental responses of honey bee antenna to a 1:10 dilution of isopentyl acetate (IPA) in mineral oil.

Fig. A3S2

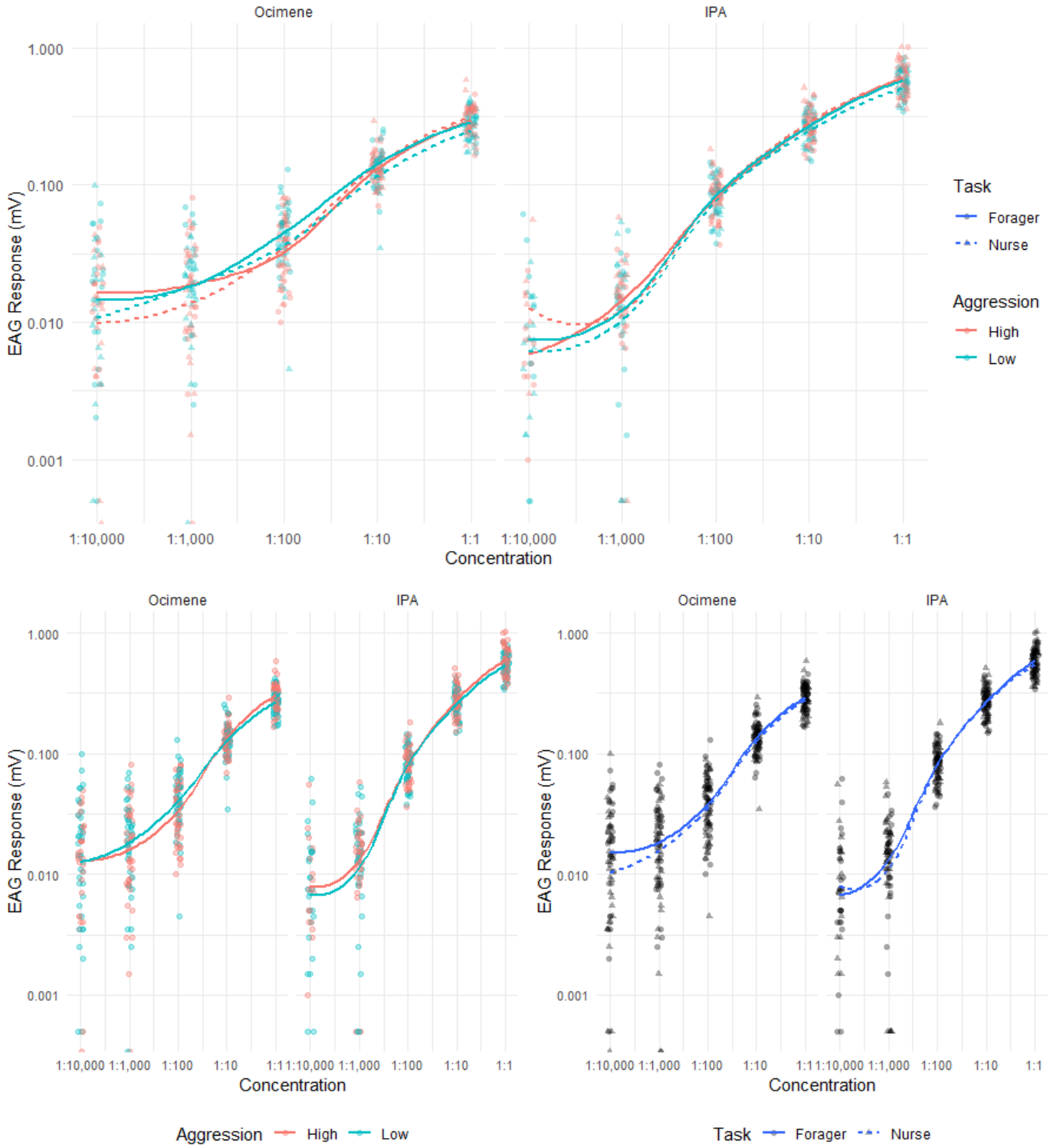

Ocimene
IPA
EAG Response (mV)
1.000
0.100
0.010
0.001
1:10,000
1:1,000
1:100
1:10
1:1
Concentration
Task
Forager
Nurse
Aggression
High
Low

Figure A3S2: Honey bees show different antennal responses to ocimene and isopentyl acetate (IPA). Top: Antennal response in millivolts to five concentrations of each odorant (from 1:10,000 to 1:1), separated by both task specialization and colony aggression. Bottom left: Same data as top, but only separating data by colony aggression. The response to ocimene was significantly affected by colony aggression when considering all concentrations. Bottom right: Same data as top, but only separating data by task specialization. The response to IPA was significantly affected by task specialization when considering all concentrations.

Fig. A3S3

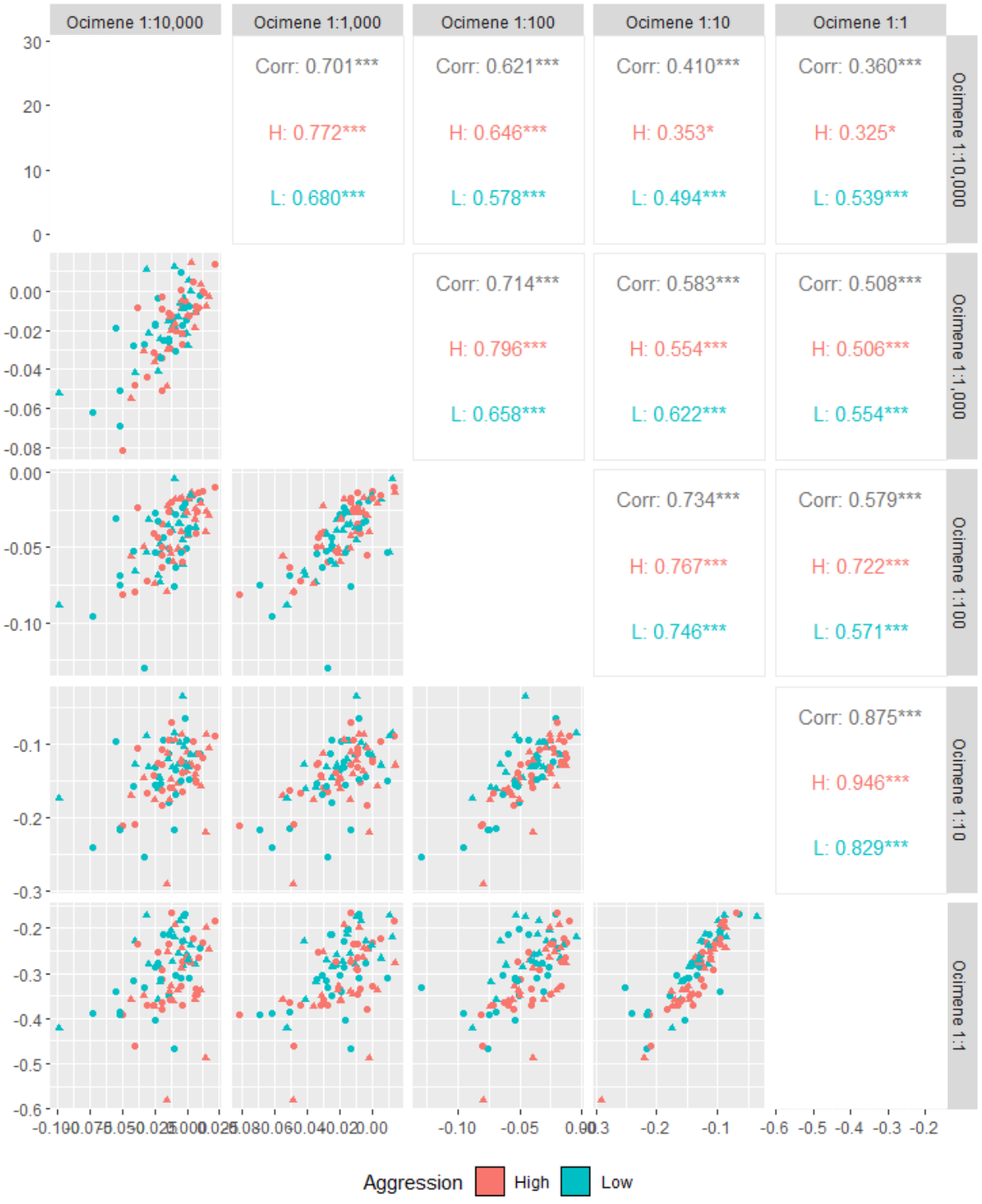

Ocimene 1:10,000
Ocimene 1:1,000
Ocimene 1:100
Ocimene 1:10
Ocimene 1:1
Corr: 0.701***
H: 0.772***
L: 0.680***
Corr: 0.621***
H: 0.646***
L: 0.578***
Corr: 0.410***
H: 0.353*
L: 0.494***
Corr: 0.360***
H: 0.325*
L: 0.539***
Corr: 0.714***
H: 0.796***
L: 0.658***
Corr: 0.583***
H: 0.554***
L: 0.622***
Corr: 0.508***
H: 0.506***
L: 0.554***
Corr: 0.734***
H: 0.767***
L: 0.746***
Corr: 0.579***
H: 0.722***
L: 0.571***
Corr: 0.875***
H: 0.946***
L: 0.829***
Aggression
High
Low

Figure A3S3: Antennal responses were significantly positively correlated between all concentrations of ocimene, with the strongest correlations being between the closest pairwise concentrations. Correlation matrix of electroantennogram response to five concentrations of ocimene, an odorant recently identified as a putative begging signal. The diagonal shows frequency distributions for each sub-group at the corresponding concentration.

Fig. A3S4

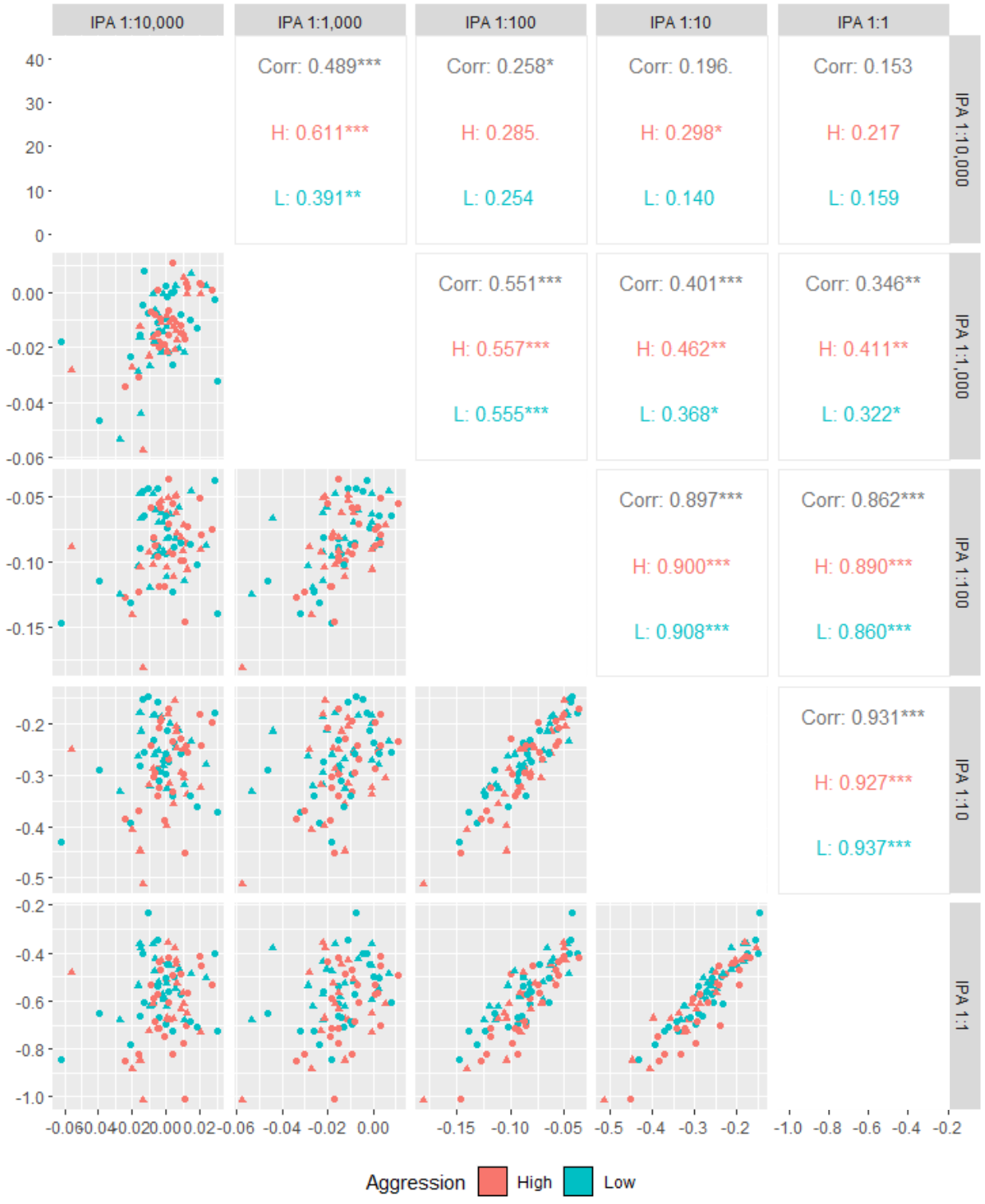

IPA 1:10,000
IPA 1:1,000
IPA 1:100
IPA 1:10
IPA 1:1
Corr: 0.489***
H: 0.611***
L: 0.391**
Corr: 0.258*
H: 0.285.
L: 0.254
Corr: 0.196.
H: 0.298*
L: 0.140
Corr: 0.153
H: 0.217
L: 0.159
Corr: 0.551***
H: 0.557***
L: 0.555***
Corr: 0.401***
H: 0.462**
L: 0.368*
Corr: 0.346**
H: 0.411**
L: 0.322*
Corr: 0.897***
H: 0.900***
L: 0.908***
Corr: 0.862***
H: 0.890***
L: 0.860***
Corr: 0.931***
H: 0.927***
L: 0.937***
Aggression
High
Low

Figure A3S4: Antennal responses were significantly positively correlated between most concentrations of isopentyl acetate (IPA), with the strongest correlations being amongst the highest three concentrations. Correlation matrix of electroantennogram response to five concentrations of IPA, an important component of honey bee alarm pheromone. The diagonal shows frequency distributions for each sub-group at the corresponding concentration.

[APPENDIX 4. Supplemental material for Chapter 5]

Table A4S1: Table displaying all primer sequences used in this study.

| **GENE NAME** | **GENBANK ID** | **PRIMER SEQUENCE** | **SOURCE** |
|---|---|---|---|
| GAPDH | GB50902 | Forward: ACTGGTATGGCCTTCCGTGTAC<br>Reverse: TGCCAAGTCTAACTGTTAAGTCAACA | (Rittschof, 2017) |
| rps5a | GB45730 | Forward: AATTATTTGGTCGCTGGAATTG<br>Reverse: TAACGTCCAGCAGAATGTGGTA | (Harrison et al., 2019) |
| tropomyosin | GB47990 | Forward: CTCGCGCTGAATTTGCTGAG<br>Reverse: GGCGAGCGACTTGTATCTGT | (Carr et al., 2020) |
| *argonaute-2* | GB50955 | Forward: AAAAAGAGCTATTGCGCGCT<br>Reverse: GGTGCCCGCCTGTACATTAA | (Brutscher et al., 2017) |
| *defensin-1* | GB41428 | Forward: TGCGCTGCTAACTGTCTCAG<br>Reverse: AATGGCACTTAACCGAAACG | (Preston et al., 2019) |
| *hopscotch* | GB44594 | Forward: TTGTGCTCCTGAAAATGCTG<br>Reverse: AACCTCCAAATCGCTCTGTG | (Chen et al., 2014) |
| *hymenoptaecin* | GB51223 | Forward: AATCGATCAGCTCTACAGACAAG<br>Reverse: ATGCAACGGCACAGAAGA | (Preston et al., 2019) |
| *PGRPSC4300* | GB15371 | Forward: GAGGCTGGTACGACATTGGT<br>Reverse: TTATAACCAGGTGCGTGTGC | (Evans et al., 2006b) |
| *ftz-f1* | GB42142 | Forward: TCTTCTCCAGATTCGAGTCCA<br>Reverse: GAAATGTTTGGCTGGGAAGA | (Mello et al., 2019) |
| *Nup98-96* | GB43238 | Forward: AAAAACCTTTTGGTACAGCAGC<br>Reverse: GACGAAGTGGAAGGTGCATTG | Novel primer created for this study. |

*Relationship between latency and expression of socially responsive genes*

Because socially responsive genes in the brain could both induce allogrooming or be affected by it, we examined the relationship between the latency to allogroom and the expression of these genes (i.e. do we see a shorter latency to groom in bees with higher expression of these genes?). We used a similar linear mixed modeling approach used in the rest of the paper, with the exception of the inclusion of latency and the interaction between behavior and latency as additional fixed effects. We found no effect of latency on the expression of either *ftz-f1* or *Nup98-96* (*ftz-f1*: ANOVA: Behavior: Wald $X^2_2$=5.8, p= 0.02, Latency: Wald $X^2_1$=0.0044, p= 0.95, Behavior*Latency interaction: Wald $X^2_2$=0.3, p= 0.85; *Nup98-96*: ANOVA: Behavior: Wald $X^2_2$=3.6, p= 0.06, Latency: Wald $X^2_1$=0.0004, p= 0.98, Behavior*Latency interaction: Wald $X^2_2$=0.3, p= 0.85; Fig. A4S1).

Fig. A4S1

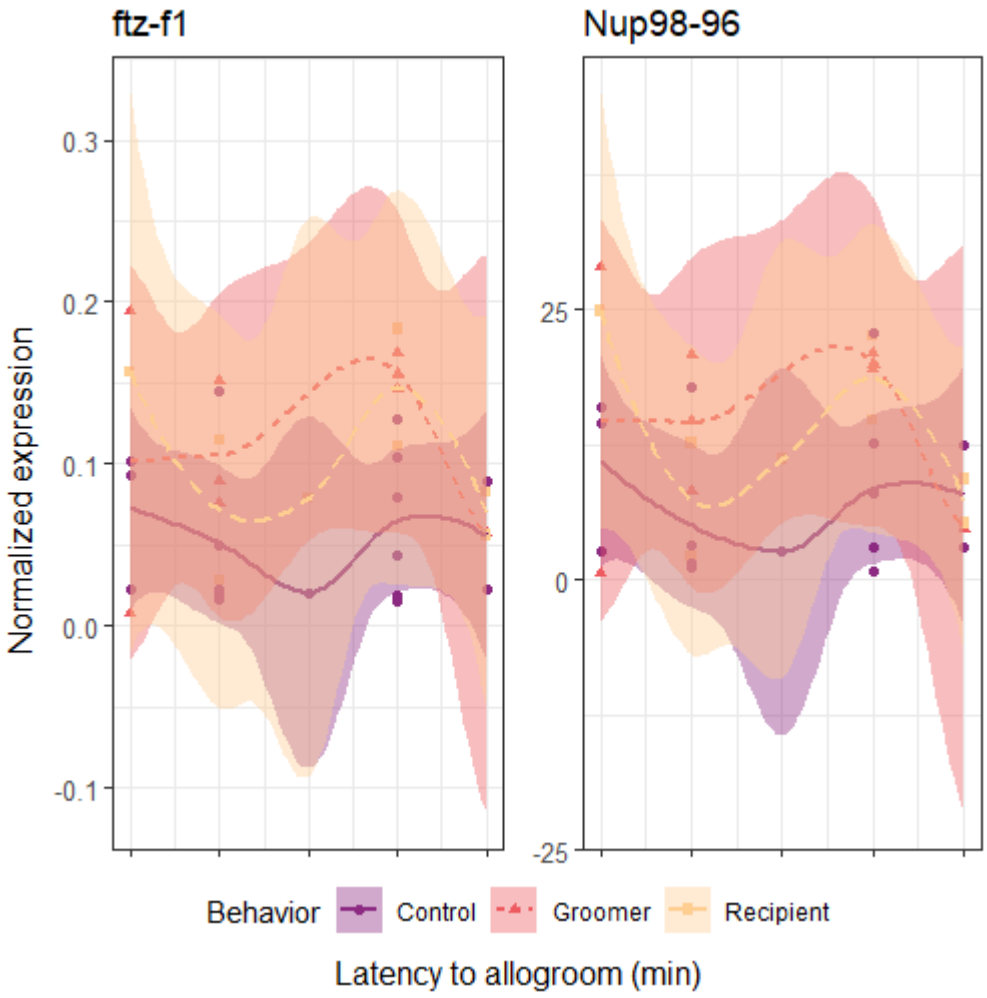



Figure A4S1: There is no relationship between the latency to allogroom and the expression of socially responsive genes *ftz-f1* or *Nup98-96* in the brains of honey bees. Scatterplot of the latency to allogroom versus normalized gene expression levels of each gene. Non-grooming controls are in dark purple, groomers are in medium red, and recipients are in light yellow.

# REFERENCES


Ahlmann-Eltze, C., & Patil, I. (2021). *ggsignif: R Package for Displaying Significance Brackets for 'ggplot2'.* In PsyArxiv.

Akhtar, Y., & Isman, M. B. (2003, 2003/08/01). Larval Exposure to Oviposition Deterrents Alters Subsequent Oviposition Behavior in Generalist, Trichoplusia ni and Specialist, Plutella xylostella Moths. *Journal of Chemical Ecology, 29*(8), 1853-1870. https://doi.org/10.1023/A:1024802328458

Akhtar, Y., Shikano, I., & Isman, M. B. (2009). Topical application of a plant extract to different life stages of Trichoplusia ni fails to influence feeding or oviposition behaviour. *Entomologia Experimentalis et Applicata, 132*(3), 275-282. https://doi.org/https://doi.org/10.1111/j.1570-7458.2009.00895.x

Alaux, C., & Robinson, G. E. (2007). Alarm pheromone induces immediate–early gene expression and slow behavioral response in honey bees. *Journal of Chemical Ecology, 33*(7), 1346-1350.

Alaux, C., Sinha, S., Hasadsri, L., Hunt, G. J., Guzmán-Novoa, E., DeGrandi-Hoffman, G., Uribe-Rubio, J. L., Southey, B. R., Rodriguez-Zas, S., & Robinson, G. E. (2009). Honey bee aggression supports a link between gene regulation and behavioral evolution. *Proceedings of the National Academy of Sciences, 106*(36), 15400-15405.

Albert, Joerg T., & Kozlov, Andrei S. (2016, 2016/10/24/). Comparative Aspects of Hearing in Vertebrates and Insects with Antennal Ears. *Current Biology, 26*(20), R1050-R1061. https://doi.org/https://doi.org/10.1016/j.cub.2016.09.017

Alburaki, M., Gregorc, A., Adamczyk, J., & Stewart, S. (2018). Insights on pollen diversity of honey bee (Apis mellifera L.) colonies located in various agricultural landscapes. *The Southwestern Naturalist, 63*(1), 49-58.

Alem, S., Perry, C. J., Zhu, X., Loukola, O. J., Ingraham, T., Søvik, E., & Chittka, L. (2016). Associative mechanisms allow for social learning and cultural transmission of string pulling in an insect. *PLOS Biology, 14*(10), e1002564.

Alfonso, S., Sadoul, B., Gesto, M., Joassard, L., Chatain, B., Geffroy, B., & Bégout, M.-L. (2019). Coping styles in European sea bass: the link between boldness, stress response and neurogenesis. *Physiology & Behavior, 207*, 76-85.

Alippi, R. M., Meta, M. D., Olivera, M. a. I., Bozzini, C., Schneider, P., Meta, I. F., & Bozzini, C. E. (2002, 2002/01/01/). Effect of protein-energy malnutrition in early life on the dimensions and bone quality of the adult rat mandible. *Archives of Oral Biology, 47*(1), 47-53. https://doi.org/https://doi.org/10.1016/S0003-9969(01)00089-9

Allan, S. A., Slessor, K. N., Winston, M. L., & King, G. G. S. (1987, 1987/01/01/). The influence of age and task specialization on the production and perception of honey bee pheromones. *Journal of Insect Physiology, 33*(12), 917-922. https://doi.org/https://doi.org/10.1016/0022-1910(87)90003-5

Alloway, T. M. (2015). Retention of Learning through Metamorphosis in the Grain Beetle Tenebrio molitor). *American Zoologist, 12*(3), 471-477. https://doi.org/10.1093/icb/12.3.471

Alvarado, S., Rajakumar, R., Abouheif, E., & Szyf, M. (2015, 2015/03/11). Epigenetic variation in the Egfr gene generates quantitative variation in a complex trait in ants. *Nature Communications, 6*(1), 6513. https://doi.org/10.1038/ncomms7513

Alyokhin, A. V., & Ferro, D. N. (1999). Modifications in dispersal and oviposition of Bt-resistant and Bt-susceptible Colorado potato beetles as a result of exposure to Bacillus thuringiensis subsp. tenebrionis Cry3A toxin. *Entomologia Experimentalis et Applicata, 90*(1), 93-101. https://doi.org/10.1046/j.1570-7458.1999.00426.x

Ament, S. A., Corona, M., Pollock, H. S., & Robinson, G. E. (2008). Insulin signaling is involved in the regulation of worker division of labor in honey bee colonies. *Proceedings of the National Academy of Sciences, 105*(11), 4226-4231. https://doi.org/10.1073/pnas.0800630105

Amigo, L., & Fontecha, J. (2011). *Milk: Goat Milk. Encycl. Dairy Sci. 484–493. doi: 10.1016*.

ANDERSON, P., & ANTON, S. (2014). Experience-based modulation of behavioural responses to plant volatiles and other sensory cues in insect herbivores. *Plant, Cell & Environment, 37*(8), 1826-1835. https://doi.org/https://doi.org/10.1111/pce.12342

Anderson, P., Sadek, M. M., Larsson, M., Hansson, B. S., & Thöming, G. (2013, 2013/06/01/). Larval host plant experience modulates both mate finding and oviposition choice in a moth. *Animal Behaviour, 85*(6), 1169-1175. https://doi.org/https://doi.org/10.1016/j.anbehav.2013.03.002

André, H. M. (1991). Stase, metamorphosis and competition in insects and other arthropods. *Belg. J. Zool, 121*(1), 3-25.

Andreas, N. J., Kampmann, B., & Mehring Le-Doare, K. (2015, 2015/11/01/). Human breast milk: A review on its composition and bioactivity. *Early Human Development, 91*(11), 629-635. https://doi.org/https://doi.org/10.1016/j.earlhumdev.2015.08.013

Ang, J. Y., Lua, J. L., Mathur, A., Thomas, R., Asmar, B. I., Savasan, S., Buck, S., Long, M., & Shankaran, S. (2012). A randomized placebo-controlled trial of massage therapy on the immune system of preterm infants. *Pediatrics, 130*(6), e1549-e1558.

Anstey, M. L., Rogers, S. M., Ott, S. R., Burrows, M., & Simpson, S. J. (2009). Serotonin Mediates Behavioral Gregarization Underlying Swarm Formation in Desert Locusts. *Science, 323*(5914), 627-630. https://doi.org/10.1126/science.1165939

Anton, S., & Rossler, W. (2020, Dec 4). Plasticity and modulation of olfactory circuits in insects. *Cell Tissue Res*. https://doi.org/10.1007/s00441-020-03329-z

Aristizabal, M. J., Anreiter, I., Halldorsdottir, T., Odgers, C. L., McDade, T. W., Goldenberg, A., Mostafavi, S., Kobor, M. S., Binder, E. B., Sokolowski, M. B., & O'Donnell, K. J. (2019, Oct 17). Biological embedding of experience: A primer on epigenetics. *Proc Natl Acad Sci U S A*. https://doi.org/10.1073/pnas.1820838116

Arsenault, S. V., Hunt, B. G., & Rehan, S. M. (2018, Aug 27). The effect of maternal care on gene expression and DNA methylation in a subsocial bee. *Nat Commun, 9*(1), 3468. https://doi.org/10.1038/s41467-018-05903-0

Arvanitakis, Z., Wang, H. Y., Capuano, A. W., Khan, A., Taib, B., Anokye-Danso, F., Schneider, J. A., Bennett, D. A., Ahima, R. S., & Arnold, S. E. (2020, Sep). Brain Insulin Signaling, Alzheimer Disease Pathology, and Cognitive Function. *Ann Neurol, 88*(3), 513-525. https://doi.org/10.1002/ana.25826

Aupinel, P., Fortini, D., Dufour, H., Tasei, J., Michaud, B., Odoux, J., & Pham-Delegue, M. (2005). Improvement of artificial feeding in a standard in vitro method for rearing Apis mellifera larvae. *Bulletin of insectology, 58*(2), 107.

Avalos, A., Fang, M., Pan, H., Lluch, A. R., Lipka, A. E., Zhao, S. D., Giray, T., Robinson, G. E., Zhang, G., & Hudson, M. E. (2020). Genomic regions influencing aggressive

behavior in honey bees are defined by colony allele frequencies. *Proceedings of the National Academy of Sciences, 117*(29), 17135-17141.

Avarguès-Weber, A. (2012, 2012/02/07/). Face Recognition: Lessons from a Wasp. *Current Biology, 22*(3), R91-R93. https://doi.org/https://doi.org/10.1016/j.cub.2011.12.040

Avishai-Eliner, S., Brunson, K. L., Sandman, C. A., & Baram, T. Z. (2002, 2002/10/01/). Stressed-out, or in (utero)? *Trends in Neurosciences, 25*(10), 518-524. https://doi.org/https://doi.org/10.1016/S0166-2236(02)02241-5

Avitsur, R., Levy, S., Goren, N., & Grinshpahet, R. (2015). Early adversity, immunity and infectious disease. *Stress, 18*(3), 289-296. https://doi.org/10.3109/10253890.2015.1017464

Awasaki, T., Lai, S.-L., Ito, K., & Lee, T. (2008). Organization and Postembryonic Development of Glial Cells in the Adult Central Brain of Drosophila. *The Journal of Neuroscience, 28*(51), 13742-13753. https://doi.org/10.1523/jneurosci.4844-08.2008

Ayup, M. M., Gärtner, P., Agosto-Rivera, J. L., Marendy, P., de Souza, P., & Galindo-Cardona, A. (2021). Analysis of Honeybee Drone Activity during the Mating Season in Northwestern Argentina. *Insects, 12*(6), 566. https://www.mdpi.com/2075-4450/12/6/566

Bachman, G. C. (1993, 1993/08/01/). The effect of body condition on the trade-off between vigilance and foraging in Belding's ground squirrels. *Animal Behaviour, 46*(2), 233-244. https://doi.org/https://doi.org/10.1006/anbe.1993.1185

Barker, D. J. (1995). Fetal origins of coronary heart disease. *Bmj, 311*(6998), 171-174.

Barron, A. (2001, 11/01). The Life and Death of Hopkins' Host-Selection Principle. *Journal of Insect Behavior, 14*, 725-737. https://doi.org/10.1023/A:1013033332535

Barron, A. B., Maleszka, R., Helliwell, P. G., & Robinson, G. E. (2008). Effects of cocaine on honey bee dance behaviour. *Journal of Experimental Biology, 212*(2), 163-168.

Bates, D. M., Martin; Bolker, Ben; Walker, Steve. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software, 67*(1), 1-48. https://doi.org/doi:10.18637/jss.v067.i01.

Beach, F. A., & Jaynes, J. (1954). Effects of early experience upon the behavior of animals. *Psychological Bulletin, 51*(3), 239-263. https://doi.org/10.1037/h0061176

Bedi, K. S., Birzgalis, A. R., Mahon, M., Smart, J. L., & Wareham, A. C. (1982). Early life undernutrition in rats: 1. Quantitative histology of skeletal muscles from underfed young and refed adult animals. *British Journal of Nutrition, 47*(3), 417-431. https://doi.org/10.1079/BJN19820053

Beekman, M., & Ratnieks, F. L. W. (2000). Long-range foraging by the honey-bee, Apis mellifera L. *Functional Ecology, 14*(4), 490-496. https://doi.org/https://doi.org/10.1046/j.1365-2435.2000.00443.x

Beekman, M., Sumpter, D., Seraphides, N., & Ratnieks, F. (2004). Comparing foraging behaviour of small and large honey-bee colonies by decoding waggle dances made by foragers. *Functional Ecology*, 829-835.

Beekman, M., Thompson, M., & Jusup, M. (2019). Thermodynamic constraints and the evolution of parental provisioning in vertebrates. *Behavioral Ecology, 30*(3), 583-591. https://doi.org/10.1093/beheco/arz025

Beery, A. K., & Francis, D. D. (2011, 2011/06/01/). Adaptive significance of natural variations in maternal care in rats: A translational perspective. *Neuroscience & Biobehavioral Reviews, 35*(7), 1552-1561. https://doi.org/https://doi.org/10.1016/j.neubiorev.2011.03.012

Beldade, P., Mateus, A. R. A., & Keller, R. A. (2011). Evolution and molecular mechanisms of adaptive developmental plasticity. *Molecular Ecology, 20*(7), 1347-1363. https://doi.org/10.1111/j.1365-294X.2011.05016.x

Bello, B. C., Izergina, N., Caussinus, E., & Reichert, H. (2008, 2008/02/19). Amplification of neural stem cell proliferation by intermediate progenitor cells in Drosophila brain development. *Neural Development, 3*(1), 5. https://doi.org/10.1186/1749-8104-3-5

Benard, M. F., & McCauley, S. J. (2008). Integrating across Life-History Stages: Consequences of Natal Habitat Effects on Dispersal. *The American Naturalist, 171*(5), 553-567. https://doi.org/10.1086/587072

Benoit, J. B. (2010). Water Management by Dormant Insects: Comparisons Between Dehydration Resistance During Summer Aestivation and Winter Diapause. In C. Arturo Navas & J. E. Carvalho (Eds.), *Aestivation: Molecular and Physiological*

*Aspects* (pp. 209-229). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-02421-4_10

Benus, R. F., Bohus, B., Koolhaas, J. M., & van Oortmerssen, G. A. (1991, 1991/10/01). Heritable variation for aggression as a reflection of individual coping strategies. *Experientia, 47*(10), 1008-1019. https://doi.org/10.1007/BF01923336

Beshers, S. N., & Fewell, J. H. (2001). Models of division of labor in social insects. *Annual Review of Entomology, 46*, 413.

Beshers, S. N., Huang, Z. Y., Oono, Y., & Robinson, G. E. (2001, 2001/12/07/). Social Inhibition and the Regulation of Temporal Polyethism in Honey Bees. *Journal of Theoretical Biology, 213*(3), 461-479. https://doi.org/https://doi.org/10.1006/jtbi.2001.2427

Betti, M. I., Wahl, L. M., & Zamir, M. (2014). Effects of Infection on Honey Bee Population Dynamics: A Model. *PLOS ONE, 9*(10), e110237. https://doi.org/10.1371/journal.pone.0110237

Betts, A. D. (1923). *Practical Bee Anatomy: With Notes on the Embryology, Metamorphoses and Physiology of the Honey Bee* (Vol. 1). Apis club.

Bicker, G., Schafer, S., Ottersen, O. P., & Storm-Mathisen, J. (1988). Glutamate-like immunoreactivity in identified neuronal populations of insect nervous systems. *Journal of Neuroscience, 8*(6), 2108-2122.

Blackiston, D. J., Silva Casey, E., & Weiss, M. R. (2008). Retention of Memory through Metamorphosis: Can a Moth Remember What It Learned As a Caterpillar? *PLOS ONE, 3*(3), e1736. https://doi.org/10.1371/journal.pone.0001736

Blumer, L. S. (1982). A bibliography and categorization of bony fishes exhibiting parental care. *Zoological journal of the Linnean Society, 75*(1), 1-22.

Boch, R., & Rothenbuhler, W. C. (1974, 1974/01/01). Defensive Behaviour and Production of Alarm Pheromone in Honeybees. *Journal of Apicultural Research, 13*(4), 217-221. https://doi.org/10.1080/00218839.1974.11099783

Boch, R., Shearer, D., & Stone, B. (1962). Identification of iso-amyl acetate as an active component in the sting pheromone of the honey bee. *Nature, 195*(4845), 1018-1020.

Boch, R., & Shearer, D. A. (1971). Chemical releasers of alarm behaviour in the honey-bee, Apis mellifera. *Journal of Insect Physiology, 17*(12), 2277-2285.

Bonner, J. T. (2004). Perspective: the size-complexity rule. *Evolution, 58*(9), 1883-1890.

Bonsels, W. (1912). *Die biene Maja und ihre abenteuer: ein roman für kinder*. Schuster & Loeffler.

Booker, R., & Truman, J. W. (1987). Postembryonic neurogenesis in the CNS of the tobacco hornworm, Manduca sexta. I. Neuroblast arrays and the fate of their progeny during metamorphosis. *Journal of Comparative Neurology, 255*(4), 548-559.

Border, S. E., DeOliveira, G. M., Janeski, H. M., Piefke, T. J., Brown, T. J., & Dijkstra, P. D. (2019). Social rank, color morph, and social network metrics predict oxidative stress in a cichlid fish. *Behavioral Ecology, 30*(2), 490-499. https://doi.org/10.1093/beheco/ary189

Bortolotti, L., & Costa, C. (2014). Chemical communication in the honey bee society. *Neurobiology of chemical communication*.

Bortolotti, L., Montanari, R., Marcelino, J., Medrzycki, P., Maini, S., & Porrini, C. (2003). Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. *Bulletin of insectology, 56*(1), 63-67.

Boyce, W. T., Sokolowski, M. B., & Robinson, G. E. (2020, Sep 22). Genes and environments, development and time. *Proc Natl Acad Sci U S A, 117*(38), 23235-23241. https://doi.org/10.1073/pnas.2016710117

Bozek, K., Hebert, L., Portugal, Y., Mikheyev, A. S., & Stephens, G. J. (2021, 2021/03/19). Markerless tracking of an entire honey bee colony. *Nature Communications, 12*(1), 1733. https://doi.org/10.1038/s41467-021-21769-1

Božič, J., & Valentinčič, T. (1995). Quantitative analysis of social grooming behavior of the honey bee Apis mellifera carnica. *Apidologie, 26*(2), 141-147.

Braaten, R. F., & Reynolds, K. (1999, 1999/07/01/). Auditory preference for conspecific song in isolation-reared zebra finches. *Animal Behaviour, 58*(1), 105-111. https://doi.org/https://doi.org/10.1006/anbe.1999.1134

Breed, M. D., Guzmán-Novoa, E., & Hunt, G. J. (2004). DEFENSIVE BEHAVIOR OF HONEY BEES: Organization, Genetics, and Comparisons with Other Bees. *Annual Review of Entomology, 49*(1), 271-298. https://doi.org/10.1146/annurev.ento.49.061802.123155

Breed, M. D., Robinson, G. E., & Page, R. E. (1990). Division of labor during honey bee colony defense. *Behavioral Ecology and Sociobiology, 27*(6), 395-401.

Breed, M. D., & Rogers, K. B. (1991). The behavioral genetics of colony defense in honeybees: genetic variability for guarding behavior. *Behavior genetics, 21*, 295-303.

Brian, M. V. (1956, 1956/09/01). Studies of caste differentiation inMyrmica rubra L. 4. Controlled larval nutrition. *Insectes Sociaux, 3*(3), 369-394. https://doi.org/10.1007/BF02225758

Brodschneider, R., & Crailsheim, K. (2010). Nutrition and health in honey bees. *Apidologie, 41*(3), 278-294.

Brodschneider, R., Riessberger-Gallé, U., & Crailsheim, K. (2009). Flight performance of artificially reared honeybees (Apis mellifera). *Apidologie, 40*(4), 441-449.

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C., Nielsen, A., KSkaug, H. J., Maechler, M., & Bolker, B. (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal, 9*(2), 378-400.

Brouwers, E. V. M., Ebert, R., & Beetsma, J. (1987, 1987/01/01). Behavioural and Physiological Aspects of Nurse Bees in Relation to the Composition of Larval Food During Caste Differentiation in the Honeybee. *Journal of Apicultural Research, 26*(1), 11-23. https://doi.org/10.1080/00218839.1987.11100729

Brown, G. E., Foam, P. E., Cowell, H. E., Fiore, P. G., & Chivers, D. P. (2004). Production of chemical alarm cues in convict cichlids: the effects of diet, body condition and ontogeny. *Annales Zoologici Fennici, 41*(3), 487-499. http://www.jstor.org/stable/23736162

Brutscher, L. M., Daughenbaugh, K. F., & Flenniken, M. L. (2017). Virus and dsRNA-triggered transcriptional responses reveal key components of honey bee antiviral defense. *Scientific Reports, 7*(1), 6448.

Bryck, R. L., & Fisher, P. A. (2012). Training the brain: practical applications of neural plasticity from the intersection of cognitive neuroscience, developmental psychology, and prevention science. *American Psychologist, 67*(2), 87.

Bubak, A. N., Watt, M. J., Yaeger, J. D. W., Renner, K. J., & Swallow, J. G. (2020, Jan 2). The stalk-eyed fly as a model for aggression - is there a conserved role for 5-HT between vertebrates and invertebrates? *J Exp Biol, 223*(Pt 1). https://doi.org/10.1242/jeb.132159

Buchinger, T. J., & Li, W. (2020, 2020/08/01). The evolution of (non)species-specific pheromones. *Evolutionary Ecology, 34*(4), 455-468. https://doi.org/10.1007/s10682-020-10046-0

Buckley, L. B., Hurlbert, A. H., & Jetz, W. (2012). Broad-scale ecological implications of ectothermy and endothermy in changing environments. *Global Ecology and Biogeography, 21*(9), 873-885. https://doi.org/10.1111/j.1466-8238.2011.00737.x

Burkett, J. P., Andari, E., Johnson, Z. V., Curry, D. C., de Waal, F. B., & Young, L. J. (2016). Oxytocin-dependent consolation behavior in rodents. *Science, 351*(6271), 375-378.

Burkhard, T. T., Westwick, R. R., & Phelps, S. M. (2018). Adiposity signals predict vocal effort in Alston's singing mice. *Proceedings of the Royal Society B: Biological Sciences, 285*(1877), 20180090. https://doi.org/doi:10.1098/rspb.2018.0090

Burrell, B. D., & Smith, B. H. (1994, 1994/05/01). Age- but not caste-related regulation of abdominal mechanisms underlying the sting reflex of the honey bee, Apis mellifera. *Journal of Comparative Physiology A, 174*(5), 581-592. https://doi.org/10.1007/BF00217379

Butler, C. G. (1954). THE METHOD AND IMPORTANCE OF THE RECOGNITION BY A COLONY OF HONEYBEES (A. MELLIFERA) OF THE PRESENCE OF ITS QUEEN. *Transactions of the Royal Entomological Society of London, 105*(2), 11-29. https://doi.org/https://doi.org/10.1111/j.1365-2311.1954.tb00773.x

Butler, J. M., & Maruska, K. P. (2020, 2020/06/01/). Underwater noise impairs social communication during aggressive and reproductive encounters. *Animal Behaviour, 164*, 9-23. https://doi.org/https://doi.org/10.1016/j.anbehav.2020.03.013

[Record #139 is using a reference type undefined in this output style.]

Calderone, N. W., & Page, R. E. (1988, 1988/01/01). Genotypic variability in age polyethism and task specialization in the honey bee, Apis mellifera (Hymenoptera: Apidae). *Behavioral Ecology and Sociobiology, 22*(1), 17-25. https://doi.org/10.1007/BF00395694

Camazine, S., Crailsheim, K., Hrassnigg, N., Robinson, G. E., Leonhard, B., & Kropiunigg, H. (1998). Protein trophallaxis and the regulation of pollen foraging by honey bees (Apis mellifera L.). *Apidologie, 29*(1-2), 113-126.

Campana, B. J., & Moeller, F. E. (1977). Honey Bees: Preference for and Nutritive Value of Pollen from Five Plant Sources123. *Journal of economic entomology, 70*(1), 39-41. https://doi.org/10.1093/jee/70.1.39

Cantera, R., Nässel, D. R., & Veenstra, J. A. (1994). Postembryonic development of corazonin-containing neurons and neurosecretory cells in the blowfly, Phormia terraenovae. *Journal of Comparative Neurology, 350*(4), 559-572. https://doi.org/10.1002/cne.903500405

Capas-Peneda, S., Saavedra Torres, Y., Prins, J.-B., & Olsson, I. A. S. (2022). From Mating to Milk Access: A Review of Reproductive Vocal Communication in Mice. *Frontiers in Behavioral Neuroscience, 16*, 833168.

Cardoso, S. D., Teles, M. C., & Oliveira, R. F. (2015, Jan 1). Neurogenomic mechanisms of social plasticity. *J Exp Biol, 218*(Pt 1), 140-149. https://doi.org/10.1242/jeb.106997

Carr, H. M., Palmer, J. H., & Rittschof, C. C. (2020). Honey bee aggression: evaluating causal links to disease-resistance traits and infection. *Behavioral Ecology and Sociobiology, 74*(9), 1-14.

Cayre, M., Malaterre, J., Charpin, P., Strambi, C., & Strambi, A. (2000, 2000/03/01/). Fate of neuroblast progeny during postembryonic development of mushroom bodies in the house cricket, Acheta domesticus. *Journal of Insect Physiology, 46*(3), 313-319. https://doi.org/https://doi.org/10.1016/S0022-1910(99)00184-5

Cayre, M., Strambi, C., & Strambi, A. (1994). Neurogenesis in an adult insect brain and its hormonal control. *Nature, 368*, 57-59.

Cejrowski, T., Szymański, J., Mora, H., & Gil, D. (2018). Detection of the bee queen presence using sound analysis. Asian Conference on Intelligent Information and Database Systems,

Champagne, F. A. (2012). Interplay between social experiences and the genome: epigenetic consequences for behavior. *Adv Genet, 77*, 33-57. https://doi.org/10.1016/B978-0-12-387687-4.00002-7

Champagne, F. A., & Curley, J. P. (2005, 2005/12/01/). How social experiences influence the brain. *Current Opinion in Neurobiology, 15*(6), 704-709. https://doi.org/https://doi.org/10.1016/j.conb.2005.10.001

Champely, S. (2018). *PairedData: Paired Data Analysis.* In (Version 1.1.1.) https://CRAN.R-project.org/package=PairedData

Chan, A. A. Y.-H., Giraldo-Perez, P., Smith, S., & Blumstein, D. T. (2010). Anthropogenic noise affects risk assessment and attention: the distracted prey hypothesis. *Biology Letters, 6*(4), 458-461. https://doi.org/doi:10.1098/rsbl.2009.1081

Chandrasekaran, S., Ament, S. A., Eddy, J. A., Rodriguez-Zas, S. L., Schatz, B. R., Price, N. D., & Robinson, G. E. (2011). Behavior-specific changes in transcriptional modules lead to distinct and predictable neurogenomic states. *Proceedings of the National Academy of Sciences, 108*(44), 18020-18025.

Chandrasekaran, S., Rittschof, C., Djukovic, D., Gu, H., Raftery, D., Price, N., & Robinson, G. (2015). Aggression is associated with aerobic glycolysis in the honey bee brain1. *Genes, Brain and Behavior, 14*(2), 158-166.

Chang, H., Ding, G., Jia, G., Feng, M., & Huang, J. (2023). Hemolymph Metabolism Analysis of Honey Bee (Apis mellifera L.) Response to Different Bee Pollens. *Insects, 14*(1), 37. https://www.mdpi.com/2075-4450/14/1/37

Charbonneau, D., & Dornhaus, A. (2015, 2015/09/01). Workers 'specialized' on inactivity: Behavioral consistency of inactive workers and their role in task allocation. *Behavioral Ecology and Sociobiology, 69*(9), 1459-1472. https://doi.org/10.1007/s00265-015-1958-1

Charbonneau, D., Sasaki, T., & Dornhaus, A. (2017). Who needs 'lazy' workers? Inactive workers act as a 'reserve' labor force replacing active workers, but inactive workers are not replaced when they are removed. *PLOS ONE, 12*(9), e0184074. https://doi.org/10.1371/journal.pone.0184074

Charles, J.-P., Iwema, T., Epa, V. C., Takaki, K., Rynes, J., & Jindra, M. (2011). Ligand-binding properties of a juvenile hormone receptor, Methoprene-tolerant. *Proceedings of the National Academy of Sciences, 108*(52), 21128-21133.

Charlesworth, B., Harvey, P. H., Godfray, H. C. J., & Johnstone, R. A. (2000). Begging and bleating: the evolution of parent–offspring signalling. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 355*(1403), 1581-1591. https://doi.org/doi:10.1098/rstb.2000.0719

Chen, Y., & Baram, T. Z. (2016). Toward understanding how early-life stress reprograms cognitive and emotional brain networks. *Neuropsychopharmacology, 41*(1), 197-206.

Chen, Y. P., Pettis, J. S., Corona, M., Chen, W. P., Li, C. J., Spivak, M., Visscher, P. K., DeGrandi-Hoffman, G., Boncristiani, H., & Zhao, Y. (2014). Israeli acute paralysis virus: epidemiology, pathogenesis and implications for honey bee health. *PLoS pathogens, 10*(7), e1004261.

Cherry, E. C. (1953). Some experiments on the recognition of speech, with one and with two ears. *The Journal of the acoustical society of America, 25*(5), 975-979.

Chittka, L., & Geiger, K. (1995, 1995/01/01/). Can honey bees count landmarks? *Animal Behaviour, 49*(1), 159-164. https://doi.org/https://doi.org/10.1016/0003-3472(95)80163-4

Chittka, L., & Niven, J. (2009, 2009/11/17/). Are Bigger Brains Better? *Current Biology, 19*(21), R995-R1008. https://doi.org/https://doi.org/10.1016/j.cub.2009.08.023

Christe, P., Oppliger, A., Bancalà, F., Castella, G., & Chapuisat, M. (2003). Evidence for collective medication in ants. *Ecology Letters, 6*(1), 19-22.

Cini, A., Bordoni, A., Cappa, F., Petrocelli, I., Pitzalis, M., Iovinella, I., Dani, F. R., Turillazzi, S., & Cervo, R. (2020, 2020/06/02). Increased immunocompetence and network centrality of allogroomer workers suggest a link between individual and social immunity in honeybees. *Scientific Reports, 10*(1), 8928. https://doi.org/10.1038/s41598-020-65780-w

Cohen, S., Janicki-Deverts, D., Turner, R. B., & Doyle, W. J. (2015). Does hugging provide stress-buffering social support? A study of susceptibility to upper respiratory infection and illness. *Psychological science, 26*(2), 135-147.

Cohn, R., Morantte, I., & Ruta, V. (2015, 2015/12/17/). Coordinated and Compartmentalized Neuromodulation Shapes Sensory Processing in Drosophila. *Cell, 163*(7), 1742-1755. https://doi.org/https://doi.org/10.1016/j.cell.2015.11.019

Cole, G. L. (2013, 2013/12/01). Lost in Translation: Adaptation of Mating Signals in Changing Environments. *Springer Science Reviews, 1*(1), 25-40. https://doi.org/10.1007/s40362-013-0009-4

Cole, S. W. (2013). Social Regulation of Human Gene Expression: Mechanisms and Implications for Public Health. *American Journal of Public Health, 103*(S1), S84-S92. https://doi.org/10.2105/ajph.2012.301183

Collins, A. M. (1980). Effect of Age on the Response to Alarm Pheromones by Caged Honey Bees. *Annals of the Entomological Society of America, 73*(3), 307-309. https://doi.org/10.1093/aesa/73.3.307

Collins, A. M., & Kubasek, K. J. (1982). Field test of honey bee (Hymenoptera: Apidae) colony defensive behavior. *Annals of the Entomological Society of America, 75*(4), 383-387.

Collins, A. M., Rinderer, T. E., Tucker, K. W., & Pesante, D. G. (1987, 1987/01/01). Response to Alarm Pheromone by European and Africanized Honeybees. *Journal of Apicultural Research, 26*(4), 217-223. https://doi.org/10.1080/00218839.1987.11100763

Collins, A. M., & Rothenbuhler, W. C. (1978). Laboratory test of the response to an alarm chemical, isopentyl acetate, by Apis tnelufera. *Annals of the Entomological Society of America, 71*(6), 906-909.

Conchou, L., Lucas, P., Meslin, C., Proffit, M., Staudt, M., & Renou, M. (2019). Insect odorscapes: from plant volatiles to natural olfactory scenes. *Frontiers in Physiology*, 972.

Conroy, T. E., & Holman, L. (2022, 2022/02/11). Social immunity in the honey bee: do immune-challenged workers enter enforced or self-imposed exile? *Behavioral Ecology and Sociobiology, 76*(2), 32. https://doi.org/10.1007/s00265-022-03139-z

Conway, A. R., Cowan, N., & Bunting, M. F. (2001). The cocktail party phenomenon revisited: The importance of working memory capacity. *Psychonomic bulletin & review, 8*, 331-335.

Coolen, I., Dangles, O., & Casas, J. (2005). Social learning in noncolonial insects? *Current Biology, 15*(21), 1931-1935.

Corbet, P. S. (1957). The life-history of the emperor dragonfly Anax imperator Leach (Odonata: Aeshnidae). *The Journal of Animal Ecology*, 1-69.

Corby-Harris, V., Snyder, L., & Meador, C. (2019, 2019/07/01/). Fat body lipolysis connects poor nutrition to hypopharyngeal gland degradation in Apis mellifera. *Journal of Insect Physiology, 116*, 1-9. https://doi.org/https://doi.org/10.1016/j.jinsphys.2019.04.001

Corby-Harris, V., Snyder, L., Meador, C., & Ayotte, T. (2018). Honey bee (Apis mellifera) nurses do not consume pollens based on their nutritional quality. *PLOS ONE, 13*(1), e0191050.

Couvillon, M. J., & Ratnieks, F. L. (2015). Environmental consultancy: Dancing bee bioindicators to evaluate landscape "health". *Frontiers in Ecology and Evolution, 3*, 44.

Couvillon, M. J., Robinson, E. J., Atkinson, B., Child, L., Dent, K. R., & Ratnieks, F. L. (2008). En garde: rapid shifts in honeybee, Apis mellifera, guarding behaviour are triggered by onslaught of conspecific intruders. *Animal Behaviour, 76*(5), 1653-1658.

Cremer, S., Armitage, S. A. O., & Schmid-Hempel, P. (2007, 2007/08/21/). Social Immunity. *Current Biology, 17*(16), R693-R702. https://doi.org/https://doi.org/10.1016/j.cub.2007.06.008

Crist, E. (2004). Can an insect speak? The case of the honeybee dance language. *Social Studies of Science, 34*(1), 7-43.

Curley, J. P., & Champagne, F. A. (2016, Jan). Influence of maternal care on the developing brain: Mechanisms, temporal dynamics and sensitive periods. *Front Neuroendocrinol, 40*, 52-66. https://doi.org/10.1016/j.yfrne.2015.11.001

D'Eath, R. B., & Lawrence, A. B. (2004, 2004/03/01/). Early life predictors of the development of aggressive behaviour in the domestic pig. *Animal Behaviour, 67*(3), 501-509. https://doi.org/https://doi.org/10.1016/j.anbehav.2003.06.010

Dall, S. R., McNamara, J. M., & Leimar, O. (2015, Jun). Genes as cues: phenotypic integration of genetic and epigenetic information from a Darwinian perspective. *Trends Ecol Evol, 30*(6), 327-333. https://doi.org/10.1016/j.tree.2015.04.002

Daly, H. V., Danka, R. G., Hoelmer, K., Rinderer, T. E., & Buco, S. M. (1995). Honey bee morphometrics: linearity of variables with respect to body size and classification tested with European worker bees reared by varying ratios of nurse bees. *Journal of Apicultural Research, 34*(3), 129-145.

Danese, A. (2020). Annual Research Review: Rethinking childhood trauma-new research directions for measurement, study design and analytical strategies. *Journal of Child Psychology and Psychiatry, 61*(3), 236-250. https://doi.org/https://doi.org/10.1111/jcpp.13160

Danihlík, J., Aronstein, K., & Petřivalský, M. (2015). Antimicrobial peptides: a key component of honey bee innate immunity: Physiology, biochemistry, and chemical ecology. *Journal of Apicultural Research, 54*(2), 123-136.

Danner, N., Keller, A., Härtel, S., & Steffan-Dewenter, I. (2017). Honey bee foraging ecology: Season but not landscape diversity shapes the amount and diversity of collected pollen. *PLOS ONE, 12*(8), e0183716.

De França, S. M., Breda, M. O., Barbosa, D. R., Araujo, A. M., & Guedes, C. A. (2017). The sublethal effects of insecticides in insects. *Biological control of pest and vector insects*, 23-39.

de Miranda, J. R., & Genersch, E. (2010, 2010/01/01/). Deformed wing virus. *Journal of Invertebrate Pathology, 103*, S48-S61. https://doi.org/https://doi.org/10.1016/j.jip.2009.06.012

De Wilde, J. (1962). Photoperiodism in insects and mites. *Annual Review of Entomology, 7*(1), 1-26.

De Wilde, J., & Beetsma, J. (1982). The Physiology of Caste Development in Social Insects. In M. J. Berridge, J. E. Treherne, & V. B. Wigglesworth (Eds.), *Advances in Insect Physiology* (Vol. 16, pp. 167-246). Academic Press. https://doi.org/https://doi.org/10.1016/S0065-2806(08)60154-X

de Zwaan, D. R., Camfield, A. F., MacDonald, E. C., & Martin, K. (2019). Variation in offspring development is driven more by weather and maternal condition than predation risk. *Functional Ecology, 33*(3), 447-456.

Dedej, S., Hartfelder, K., Aumeier, P., Rosenkranz, P., & Engels, W. (1998, 1998/01/01). Caste determination is a sequential process: effect of larval age at grafting on ovariole number, hind leg size and cephalic volatiles in the honey bee (Apis mellifera carnica). *Journal of Apicultural Research, 37*(3), 183-190. https://doi.org/10.1080/00218839.1998.11100970

Deisig, N., Dupuy, F., Anton, S., & Renou, M. (2014). Responses to Pheromones in a Complex Odor World: Sensory Processing and Behavior. *Insects, 5*(2), 399-422. https://www.mdpi.com/2075-4450/5/2/399

Demartsev, V., Gersick, A. S., Jensen, F. H., Thomas, M., Roch, M. A., Manser, M. B., & Strandburg-Peshkin, A. Signalling in groups: New tools for the integration of animal communication and collective movement. *Methods in Ecology and Evolution, n/a*(n/a). https://doi.org/https://doi.org/10.1111/2041-210X.13939

Deobagkar, D. (2018, 2018/06/01). Epigenetics with special reference to the human X chromosome inactivation and the enigma of Drosophila DNA methylation. *Journal of Genetics, 97*(2), 371-378. https://doi.org/10.1007/s12041-018-0937-5

Deshmukh, S., Ponnaluri, V. C., Dai, N., Pradhan, S., & Deobagkar, D. (2018). Levels of DNA cytosine methylation in the Drosophila genome. *PeerJ, 6*, e5119.

Desneux, N., Decourtye, A., & Delpuech, J.-M. (2007). The Sublethal Effects of Pesticides on Beneficial Arthropods. *Annual Review of Entomology, 52*(1), 81-106. https://doi.org/10.1146/annurev.ento.52.110405.091440

Di Prisco, G., Cavaliere, V., Annoscia, D., Varricchio, P., Caprio, E., Nazzi, F., Gargiulo, G., & Pennacchio, F. (2013). Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. *Proceedings of the National Academy of Sciences, 110*(46), 18466-18471. https://doi.org/10.1073/pnas.1314923110

Di Sante, J., Ismaylova, E., Nemoda, Z., Gouin, J. P., Yu, W. J., Caldwell, W., Vitaro, F., Szyf, M., Tremblay, R. E., & Booij, L. (2018, Nov). Peripheral DNA methylation of HPA axis-related genes in humans: Cross-tissue convergence, two-year stability and behavioural and neural correlates. *Psychoneuroendocrinology, 97*, 196-205. https://doi.org/10.1016/j.psyneuen.2018.07.019

Doherty, A. M., Lodge, C. J., Dharmage, S. C., Dai, X., Bode, L., & Lowe, A. J. (2018). Human milk oligosaccharides and associations with immune-mediated disease and infection in childhood: a systematic review. *Frontiers in Pediatrics, 6*, 91.

Donkersley, P., Rhodes, G., Pickup, R. W., Jones, K. C., Power, E. F., Wright, G. A., & Wilson, K. (2017, 2017/12/01). Nutritional composition of honey bee food stores vary with floral composition. *Oecologia, 185*(4), 749-761. https://doi.org/10.1007/s00442-017-3968-3

Downs, S. G., & Ratnieks, F. L. W. (2000). Adaptive shifts in honey bee (Apis mellifera L.) guarding behavior support predictions of the acceptance threshold model. *Behavioral Ecology, 11*(3), 326-333. https://doi.org/10.1093/beheco/11.3.326

Duffey, E. (1951). FIELD STUDIES ON THE FULMAR FULMARUS GLACIALIS. *Ibis, 93*(2), 237-245. https://doi.org/https://doi.org/10.1111/j.1474-919X.1951.tb05421.x

Dunbar, R. I. M. (2010, 2010/02/01/). The social role of touch in humans and primates: Behavioural function and neurobiological mechanisms. *Neuroscience & Biobehavioral Reviews, 34*(2), 260-268. https://doi.org/https://doi.org/10.1016/j.neubiorev.2008.07.001

Dworkin, I., & Bolker, B. (2021). *Multivariate modeling via mixed models*. https://mac-theobio.github.io/QMEE/lectures/MultivariateMixed.notes.html#a-trick-to-do-multivariate-mixed-models-using-lme4

Dyer, F. C. (1998). 5 - Spatial Cognition: Lessons from Central-place Foraging Insects. In R. P. Balda, I. M. Pepperberg, & A. C. Kamil (Eds.), *Animal Cognition in Nature* (pp. 119-154). Academic Press. https://doi.org/https://doi.org/10.1016/B978-012077030-4/50057-X

Ebenman, B. (1992). Evolution in organisms that change their niches during the life cycle. *The American Naturalist, 139*(5), 990-1021.

Eichmütler, S., & Schäfer, S. (1995). Sensory neuron development revealed by taurine immunocytochemistry in the honeybee. *Journal of Comparative Neurology, 352*(2), 297-307. https://doi.org/https://doi.org/10.1002/cne.903520211

Eischen, F. A., Rothenbuhler, W. C., & Kulinčević, J. M. (1982, 1982/01/01). Length of life and Dry Weight of Worker Honeybees Reared in Colonies with Different Worker-Larva Ratios. *Journal of Apicultural Research, 21*(1), 19-25. https://doi.org/10.1080/00218839.1982.11100511

Eischen, F. A., Rothenbuhler, W. C., & Kulinčević, J. M. (1983, 1983/01/01). Brood Rearing Associated with a Range of Worker-Larva Ratios in the Honeybee. *Journal of Apicultural Research, 22*(3), 163-168. https://doi.org/10.1080/00218839.1983.11100582

Eisenberger, N. I., Moieni, M., Inagaki, T. K., Muscatell, K. A., & Irwin, M. R. (2017, 2017/01/01). In Sickness and in Health: The Co-Regulation of Inflammation and Social Behavior. *Neuropsychopharmacology, 42*(1), 242-253. https://doi.org/10.1038/npp.2016.141

Eishchen, F. A., Rothenbuhler, W. C., & Kulinčević, J. M. (1982). Length of life and dry weight of worker honeybees reared in colonies with different worker-larva ratios. *Journal of Apicultural Research, 21*(1), 19-25.

Eleftherianos, I., Mohamed, A. A., Tettamanti, G., & Zhang, W. (2023). Insect behavioral adaptations and immune responses to stress. *Frontiers in Physiology, 14*, 1244589.

Elekonich, M. M., & Robinson, G. E. (2000, 2000/12/01/). Organizational and activational effects of hormones on insect behavior. *Journal of Insect Physiology, 46*(12), 1509-1515. https://doi.org/https://doi.org/10.1016/S0022-1910(00)00101-3

Elwood, R. W., Wood, K. E., Gallagher, M. B., & Dick, J. T. A. (1998, 1998/05/01). Probing motivational state during agonistic encounters in animals. *Nature, 393*(6680), 66-68. https://doi.org/10.1038/29980

Emlen, D. J. (1997, 1997/11/01). Alternative reproductive tactics and male-dimorphism in the horned beetle Onthophagus acuminatus (Coleoptera: Scarabaeidae). *Behavioral Ecology and Sociobiology, 41*(5), 335-341. https://doi.org/10.1007/s002650050393

Endler, J. A., Butlin, R. K., Guilford, T., & Krebs, J. R. (1993). Some general comments on the evolution and design of animal communication systems. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 340*(1292), 215-225. https://doi.org/doi:10.1098/rstb.1993.0060

Erion, R., & Sehgal, A. (2013, 2013-December-03). Regulation of insect behavior via the insulin-signaling pathway [Mini Review]. *Frontiers in Physiology, 4*(353). https://doi.org/10.3389/fphys.2013.00353

Evans, J. D., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J.-L., Jiang, H., Kanost, M., Thompson, G. J., Zou, Z., & Hultmark, D. (2006a). Immune pathways and defence

mechanisms in honey bees Apis mellifera. *Insect molecular biology, 15*(5), 645-656. https://doi.org/https://doi.org/10.1111/j.1365-2583.2006.00682.x

Evans, J. D., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J. L., Jiang, H., Kanost, M., Thompson, G. J., Zou, Z., & Hultmark, D. (2006b). Immune pathways and defence mechanisms in honey bees Apis mellifera. *Insect molecular biology, 15*(5), 645-656. https://doi.org/10.1111/j.1365-2583.2006.00682.x

Ezenwa, V. O., Gerardo, N. M., Inouye, D. W., Medina, M., & Xavier, J. B. (2012). Animal behavior and the microbiome. *Science, 338*(6104), 198-199.

Fagundes, C. P., Glaser, R., & Kiecolt-Glaser, J. K. (2013, 2013/01/01/). Stressful early life experiences and immune dysregulation across the lifespan. *Brain, Behavior, and Immunity, 27*, 8-12. https://doi.org/https://doi.org/10.1016/j.bbi.2012.06.014

Fahrbach, S. E. (2006). STRUCTURE OF THE MUSHROOM BODIES OF THE INSECT BRAIN. *Annual Review of Entomology, 51*(1), 209-232. https://doi.org/10.1146/annurev.ento.51.110104.150954

Fahrbach, S. E., Moore, D., Capaldi, E. A., Farris, S. M., & Robinson, G. E. (1998). Experience-expectant plasticity in the mushroom bodies of the honeybee. *Learning & Memory, 5*(1), 115-123.

Farina, W. M., Balbuena, M. S., Herbert, L. T., Mengoni Goñalons, C., & Vázquez, D. E. (2019). Effects of the Herbicide Glyphosate on Honey Bee Sensory and Cognitive Abilities: Individual Impairments with Implications for the Hive. *Insects, 10*(10), 354. https://www.mdpi.com/2075-4450/10/10/354

Farris, S. M. (2008). Structural, Functional and Developmental Convergence of the Insect Mushroom Bodies with Higher Brain Centers of Vertebrates. *Brain, Behavior and Evolution, 72*(1), 1-15. https://doi.org/10.1159/000139457

Favaro, R., Roved, J., Haase, A., & Angeli, S. (2022). Impact of chronic exposure to two neonicotinoids on honey bee antennal responses to flower volatiles and pheromonal compounds. *Frontiers in Insect Science, 2*.

Fawcett, T. W., Fallenstein, B., Higginson, A. D., Houston, A. I., Mallpress, D. E. W., Trimmer, P. C., & McNamara, J. M. (2014, 2014/03/01/). The evolution of decision rules in complex environments. *Trends in cognitive sciences, 18*(3), 153-161. https://doi.org/https://doi.org/10.1016/j.tics.2013.12.012

Felger, J. C., & Treadway, M. T. (2017, 2017/01/01). Inflammation Effects on Motivation and Motor Activity: Role of Dopamine. *Neuropsychopharmacology, 42*(1), 216-241. https://doi.org/10.1038/npp.2016.143

Felsenberg, J., Jacob, P. F., Walker, T., Barnstedt, O., Edmondson-Stait, A. J., Pleijzier, M. W., Otto, N., Schlegel, P., Sharifi, N., Perisse, E., Smith, C. S., Lauritzen, J. S., Costa, M., Jefferis, G. S. X. E., Bock, D. D., & Waddell, S. (2018, 2018/10/18/). Integration of Parallel Opposing Memories Underlies Memory Extinction. *Cell, 175*(3), 709-722.e715. https://doi.org/https://doi.org/10.1016/j.cell.2018.08.021

Feng, S., Cokus, S. J., Zhang, X., Chen, P.-Y., Bostick, M., Goll, M. G., Hetzel, J., Jain, J., Strauss, S. H., Halpern, M. E., Ukomadu, C., Sadler, K. C., Pradhan, S., Pellegrini, M., & Jacobsen, S. E. (2010). Conservation and divergence of methylation patterning in plants and animals. *Proceedings of the National Academy of Sciences, 107*(19), 8689-8694. https://doi.org/10.1073/pnas.1002720107

Ferguson, A. W., & Free, J. B. (1980). Queen pheromone transfer within honeybee colonies. *Physiological Entomology, 5*(4), 359-366. https://doi.org/https://doi.org/10.1111/j.1365-3032.1980.tb00245.x

Fernandez, M. P., Pettibone, H. L., Bogart, J. T., Roell, C. J., Davey, C. E., Pranevicius, A., Huynh, K. V., Lennox, S. M., Kostadinov, B. S., & Shafer, O. T. (2020, Jun 22). Sites of Circadian Clock Neuron Plasticity Mediate Sensory Integration and Entrainment. *Curr Biol, 30*(12), 2225-2237 e2225. https://doi.org/10.1016/j.cub.2020.04.025

Fikadu, Z. (2019). The contribution of managed honey bees to crop pollination, food security, and economic stability: Case of Ethiopia. *The Open Agriculture Journal, 13*(1).

Fischman, B. J., Woodard, S. H., & Robinson, G. E. (2011). Molecular evolutionary analyses of insect societies. *Proceedings of the National Academy of Sciences, 108*(Supplement 2), 10847-10854.

Flatt, T., Moroz, L. L., Tatar, M., & Heyland, A. (2006). Comparing thyroid and insect hormone signaling. *Integrative and Comparative Biology, 46*(6), 777-794. https://doi.org/10.1093/icb/icl034

Flores, K., Wolschin, F., Corneveaux, J. J., Allen, A. N., Huentelman, M. J., & Amdam, G. V. (2012, 2012/09/15). Genome-wide association between DNA methylation and alternative splicing in an invertebrate. *BMC Genomics, 13*(1), 480. https://doi.org/10.1186/1471-2164-13-480

Flynn, A. M., & Smee, D. L. (2010, 2010/01/31/). Behavioral plasticity of the soft-shell clam, Mya arenaria (L.), in the presence of predators increases survival in the field. *Journal of Experimental Marine Biology and Ecology, 383*(1), 32-38. https://doi.org/https://doi.org/10.1016/j.jembe.2009.10.017

Foray, V., Pelisson, P.-F., Bel-Venner, M.-C., Desouhant, E., Venner, S., Menu, F., Giron, D., & Rey, B. (2012). A handbook for uncovering the complete energetic budget in insects: the van Handel's method (1985) revisited. *Physiological Entomology, 37*(3), 295-302. https://doi.org/https://doi.org/10.1111/j.1365-3032.2012.00831.x

Fox, J., & Weisberg, S. (2019). *An {R} Companion to Applied Regression, Third Edition.* .

Frasnelli, E., Anfora, G., Trona, F., Tessarolo, F., & Vallortigara, G. (2010, 2010/06/19/). Morpho-functional asymmetry of the olfactory receptors of the honeybee (Apis mellifera). *Behavioural Brain Research, 209*(2), 221-225. https://doi.org/https://doi.org/10.1016/j.bbr.2010.01.046

Gabbutt, P. D. (1959). The bionomics of the wood cricket, Nemobius sylvestris (Orthoptera: Gryllidae). *The Journal of Animal Ecology*, 15-42.

Gadagkar, R., Bhagavan, S., Chandrashekara, K., & Vinutha, C. (1991). The role of larval nutrition in pre-imaginal biasing of caste in the primitively eusocial wasp Ropalidia marginata(Hymenoptera: Vespidae). *Ecological entomology, 16*(4), 435-440.

Gadagkar, V., Puzerey, P. A., Chen, R., Baird-Daniel, E., Farhang, A. R., & Goldberg, J. H. (2016). Dopamine neurons encode performance error in singing birds. *Science, 354*(6317), 1278-1282.

Galbraith, D. A., Yang, X., Nino, E. L., Yi, S., & Grozinger, C. (2015). Parallel epigenomic and transcriptomic responses to viral infection in honey bees (Apis mellifera). *PLoS pathogens, 11*(3), e1004713.

Garbuzov, M., Balfour, N. J., Shackleton, K., Al Toufailia, H., Scandian, L., & Ratnieks, F. L. W. (2020). Multiple methods of assessing nectar foraging conditions indicate peak foraging difficulty in late season. *Insect Conservation and Diversity, 13*(6), 532-542. https://doi.org/https://doi.org/10.1111/icad.12420

Garnier, S., Ross, N., Rudis, R., Camargo, A. P., Sciaini, M., & Scherer, C. (2021). *Rvision - Colorblind-Friendly Color Maps for R.* In (Version 0.6.2) R package.

Ghosh, S., Jeon, H., & Jung, C. (2020, 2020/01/21). Foraging behaviour and preference of pollen sources by honey bee (Apis mellifera) relative to protein contents. *Journal of Ecology and Environment, 44*(1), 4. https://doi.org/10.1186/s41610-020-0149-9

Gibson, G. (2008, 2008/08/01). The environmental contribution to gene expression profiles. *Nature Reviews Genetics, 9*(8), 575-581. https://doi.org/10.1038/nrg2383

Gil, A., & Sanchez-Medina, F. (1981). Acid-soluble nucleotides of cow's, goat's and sheep's milks, at different stages of lactation. *Journal of Dairy Research, 48*(1), 35-44. https://doi.org/10.1017/S0022029900021427

Gilbert, L. I., Tata, J. R., & Atkinson, B. G. (1996). *Metamorphosis: postembryonic reprogramming of gene expression in amphibian and insect cells*. Academic Press.

Gillett, S. D. (1973, 1973/08/01/). Social determinants of aggregation behaviour in adults of the desert locust. *Animal Behaviour, 21*(3), 599-606. https://doi.org/https://doi.org/10.1016/S0003-3472(73)80022-3

Gilliam, M., TABER III, S., & Richardson, G. V. (1983). Hygienic behavior of honey bees in relation to chalkbrood disease. *Apidologie, 14*(1), 29-39.

Giurfa, M., Eichmann, B., & Menzel, R. (1996). Symmetry perception in an insect. *Nature, 382*(6590), 458-461.

Giurfa, M., Zhang, S., Jenett, A., Menzel, R., & Srinivasan, M. V. (2001). The concepts of 'sameness' and 'difference'in an insect. *Nature, 410*(6831), 930-933.

Glastad, K. M., Hunt, B. G., & Goodisman, M. A. D. (2014, 2014/07/01/). Evolutionary insights into DNA methylation in insects. *Current Opinion in Insect Science, 1*, 25-30. https://doi.org/https://doi.org/10.1016/j.cois.2014.04.001

Glastad, K. M., Hunt, B. G., & Goodisman, M. A. D. (2019, Jan 7). Epigenetics in Insects: Genome Regulation and the Generation of Phenotypic Diversity. *Annual Review of Entomology, 64*(1), 185-203. https://doi.org/10.1146/annurev-ento-011118-111914

Glavinic, U., Tesovnik, T., Stevanovic, J., Zorc, M., Cizelj, I., Stanimirovic, Z., & Narat, M. (2019). Response of adult honey bees treated in larval stage with prochloraz to infection with Nosema ceranae. *PeerJ, 7*, e6325.

Gomes, D. G., & Goerlitz, H. R. (2020). Individual differences show that only some bats can cope with noise-induced masking and distraction. *PeerJ, 8*, e10551.

Gontarski, H. (1953). Zur Brutbiologie der Honigbiene. *Z. Bienenforsch, 2*(1), 7-10.

Gonzalez-Tokman, D., Cordoba-Aguilar, A., Dattilo, W., Lira-Noriega, A., Sanchez-Guillen, R. A., & Villalobos, F. (2020, Jun). Insect responses to heat: physiological mechanisms, evolution and ecological implications in a warming world. *Biol Rev Camb Philos Soc, 95*(3), 802-821. https://doi.org/10.1111/brv.12588

Grace, J. L., & Shaw, K. L. (2004). EFFECTS OF DEVELOPMENTAL ENVIRONMENT ON SIGNAL-PREFERENCE COUPLING IN A HAWAIIAN CRICKET. *Evolution, 58*(7), 1627-1633. https://doi.org/10.1111/j.0014-3820.2004.tb01744.x

Grgurevic, N., Büdefeld, T., Rissman, E. F., Tobet, S. A., & Majdic, G. (2008). Aggressive behaviors in adult SF-1 knockout mice that are not exposed to gonadal steroids during development. *Behavioral Neuroscience, 122*(4), 876-884. https://doi.org/10.1037/0735-7044.122.4.876

Groothuis, J., & Smid, H. M. (2017). Nasonia parasitic wasps escape from Haller's rule by diphasic, partially isometric brain-body size scaling and selective neuropil adaptations. *Brain, Behavior and Evolution, 90*(3), 243-254.

Gross, K., Pasinelli, G., & Kunc, Hansjoerg P. (2010). Behavioral Plasticity Allows Short-Term Adjustment to a Novel Environment. *The American Naturalist, 176*(4), 456-464. https://doi.org/10.1086/655428

Groves, P. M., Lee, D., & Thompson, R. F. (1969). Effects of stimulus frequency and intensity on habituation and sensitization in acute spinal cat. *Physiology & Behavior, 4*(3), 383-388.

Grueter, C. C., Bissonnette, A., Isler, K., & van Schaik, C. P. (2013, 2013/01/01/). Grooming and group cohesion in primates: implications for the evolution of language. *Evolution and Human Behavior, 34*(1), 61-68. https://doi.org/https://doi.org/10.1016/j.evolhumbehav.2012.09.004

Guthrie, H. A., & Brown, M. L. (1968). Effect of Severe Undernutrition in Early Life on Growth, Brain Size and Composition in Adult Rats. *The Journal of Nutrition, 94*(4), 419-426. https://doi.org/10.1093/jn/94.4.419

Gutierrez, D. V., Oh, E., & Herlitze, S. (2011). Vertebrate and Invertebrate Rhodopsins: Light Control of G-Protein Signaling. In *Photosensitive Molecules for Controlling Biological Function* (pp. 133-146). Springer.

Guzmán-Novoa, E., Hunt, G. J., Uribe-Rubio, J. L., & Prieto-Merlos, D. (2004). Genotypic effects of honey bee (Apis mellifera) defensive behavior at the individual and colony levels: the relationship of guarding, pursuing and stinging. *Apidologie, 35*(1), 15-24.

Guzman-Novoa, E., & Page, R. E. (1994). Genetic dominance and worker interactions affect honeybee colony defense. *Behavioral Ecology, 5*(1), 91-97.

Guzmán-Novoa, E., Prieto-Merlos, D., Uribe-Rubio, J. L., & Hunt, G. J. (2003). Relative reliability of four field assays to test defensive behaviour of honey bees (Apis mellifera). *Journal of Apicultural Research, 42*(3), 42-46.

Hagler, J. R., Mueller, S., Teuber, L. R., Machtley, S. A., & Van Deynze, A. (2011). Foraging range of honey bees, Apis mellifera, in alfalfa seed production fields. *Journal of Insect Science, 11*(1). https://doi.org/10.1673/031.011.14401

Hahn-Holbrook, J., Dunkel Schetter, C., Arora, C., & Hobel, C. J. (2013). Placental Corticotropin-Releasing Hormone Mediates the Association Between Prenatal Social Support and Postpartum Depression. *Clinical Psychological Science, 1*(3), 253-265. https://doi.org/10.1177/2167702612470646

Hähnlein, I., & Bicker, G. (1997, 1997/05/01). Glial patterning during postembryonic development of central neuropiles in the brain of the honeybee. *Development Genes and Evolution, 207*(1), 29-41. https://doi.org/10.1007/s004270050089

Hall, Z. J., & Tropepe, V. (2020). Using teleost fish to discern developmental signatures of evolutionary adaptation from phenotypic plasticity in brain structure. *Frontiers in neuroanatomy, 14*, 10.

Hamiduzzaman, M. M., Emsen, B., Hunt, G. J., Subramanyam, S., Williams, C. E., Tsuruda, J. M., & Guzman-Novoa, E. (2017, 2017/05/01). Differential Gene Expression Associated with Honey Bee Grooming Behavior in Response to Varroa Mites. *Behavior genetics, 47*(3), 335-344. https://doi.org/10.1007/s10519-017-9834-6

Han, B., Fang, Y., Feng, M., Hu, H., Qi, Y., Huo, X., Meng, L., Wu, B., & Li, J. (2015). Quantitative neuropeptidome analysis reveals neuropeptides are correlated with

social behavior regulation of the honeybee workers. *Journal of proteome research, 14*(10), 4382-4393.

Han, C. S., & Brooks, R. C. (2014). Long-Term Effect of Social Interactions on Behavioral Plasticity and Lifetime Mating Success. *The American Naturalist, 183*(3), 431-444. https://doi.org/10.1086/674935

Harbo, J. R. (1986, 1986/01/01). Effect of Population Size on Brood Production, Worker Survival and Honey Gain in Colonies of Honeybees. *Journal of Apicultural Research, 25*(1), 22-29. https://doi.org/10.1080/00218839.1986.11100687

Hardin, J. W., Hardin, J. W., Hilbe, J. M., & Hilbe, J. (2007). *Generalized linear models and extensions*. Stata press.

Haroon, E., Miller, A. H., & Sanacora, G. (2017, 2017/01/01). Inflammation, Glutamate, and Glia: A Trio of Trouble in Mood Disorders. *Neuropsychopharmacology, 42*(1), 193-215. https://doi.org/10.1038/npp.2016.199

Harpur, B. A., Kadri, S. M., Orsi, R. O., Whitfield, C. W., & Zayed, A. (2020). Defense response in Brazilian honey bees (Apis mellifera scutellata× spp.) is underpinned by complex patterns of admixture. *Genome Biology and Evolution, 12*(8), 1367-1377.

Harrison, J. W., Palmer, J. H., & Rittschof, C. C. (2019, 2019/10/10). Altering social cue perception impacts honey bee aggression with minimal impacts on aggression-related brain gene expression. *Scientific Reports, 9*(1), 14642. https://doi.org/10.1038/s41598-019-51223-8

Hart, B. L. (1990). Behavioral adaptations to pathogens and parasites: five strategies. *Neuroscience & Biobehavioral Reviews, 14*(3), 273-294.

Hart, B. L., & Hart, L. A. (2018). How mammals stay healthy in nature: the evolution of behaviours to avoid parasites and pathogens. *Philosophical Transactions of the Royal Society B: Biological Sciences, 373*(1751), 20170205. https://doi.org/doi:10.1098/rstb.2017.0205

Hartig, F. (2022). *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models.* In (Version version 0.4.5) R package. https://CRAN.R-project.org/package=DHARMa

Hattori, D., Aso, Y., Swartz, K. J., Rubin, G. M., Abbott, L. F., & Axel, R. (2017, 2017/05/18/). Representations of Novelty and Familiarity in a Mushroom Body Compartment. *Cell, 169*(5), 956-969.e917. https://doi.org/https://doi.org/10.1016/j.cell.2017.04.028

Hattori, Y., Kano, F., & Tomonaga, M. (2010). Differential sensitivity to conspecific and allospecific cues in chimpanzees and humans: a comparative eye-tracking study. *Biology Letters, 6*(5), 610-613. https://doi.org/doi:10.1098/rsbl.2010.0120

Haydak, M. (1935). Brood rearing by honey bees confined to a pure carbohydrate diet. *Journal of economic entomology, 28*, 657-660.

Haynes, K. F. (1988). Sublethal effects of neurotoxic insecticides on insect behavior. *Annual Review of Entomology, 33*(1), 149-168.

He, X. J., Zhang, X. C., Jiang, W. J., Barron, A. B., Zhang, J. H., & Zeng, Z. J. (2016, 2016/02/29). Starving honey bee (Apis mellifera) larvae signal pheromonally to worker bees. *Scientific Reports, 6*(1), 22359. https://doi.org/10.1038/srep22359

Heimken, C., Aumeier, P., & Kirchner, W. H. (2009). Mechanisms of food provisioning of honeybee larvae by worker bees. *Journal of Experimental Biology, 212*(7), 1032-1035.

Heinzeller, T., Joshi, B., Nürnberger, F., & Reiter, R. (1988). Effects of aggressive encounters on pineal melatonin formation in male gerbils (Meriones unguiculatus, Cricetidae). *Journal of Comparative Physiology A, 164*, 91-94.

Heisenberg, M., Heusipp, M., & Wanke, C. (1995). Structural plasticity in the Drosophila brain. *The Journal of Neuroscience, 15*(3), 1951-1960. https://doi.org/10.1523/jneurosci.15-03-01951.1995

Henazi, S. P., & Barrett, L. (1999, 1999/01/01). The value of grooming to female primates. *Primates, 40*(1), 47-59. https://doi.org/10.1007/BF02557701

Hendriksma, H. P., & Shafir, S. (2016, 2016/04/01). Honey bee foragers balance colony nutritional deficiencies. *Behavioral Ecology and Sociobiology, 70*(4), 509-517. https://doi.org/10.1007/s00265-016-2067-5

Hensch, T. K. (2004). CRITICAL PERIOD REGULATION. *Annual Review of Neuroscience, 27*(1), 549-579. https://doi.org/10.1146/annurev.neuro.27.070203.144327

Herb, B. R., Wolschin, F., Hansen, K. D., Aryee, M. J., Langmead, B., Irizarry, R., Amdam, G. V., & Feinberg, A. P. (2012, Oct). Reversible switching between epigenetic states in honeybee behavioral subcastes. *Nat Neurosci, 15*(10), 1371-1373. https://doi.org/10.1038/nn.3218

Herberstein, M. E., Wignall, A. E., Hebets, E. A., & Schneider, J. M. (2014, 2014/10/01/). Dangerous mating systems: Signal complexity, signal content and neural capacity in spiders. *Neuroscience & Biobehavioral Reviews, 46*, 509-518. https://doi.org/https://doi.org/10.1016/j.neubiorev.2014.07.018

Heyland, A., & Moroz, L. L. (2006). Signaling mechanisms underlying metamorphic transitions in animals. *Integrative and Comparative Biology, 46*(6), 743-759. https://doi.org/10.1093/icb/icl023

Hillegass, M. A., Waterman, J. M., & Roth, J. D. (2010). Parasite removal increases reproductive success in a social African ground squirrel. *Behavioral Ecology, 21*(4), 696-700.

Hirata, M., & Higashi, S. (2008, 2008/02/22). Degree-day accumulation controlling allopatric and sympatric variations in the sociality of sweat bees, Lasioglossum (Evylaeus) baleicum (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology, 62*(8), 1239. https://doi.org/10.1007/s00265-008-0552-1

Hobert, O. (2003). Behavioral plasticity in C. elegans: Paradigms, circuits, genes. *Journal of Neurobiology, 54*(1), 203-223. https://doi.org/https://doi.org/10.1002/neu.10168

Hochberg, Z. e., Feil, R., Constancia, M., Fraga, M., Junien, C., Carel, J.-C., Boileau, P., Le Bouc, Y., Deal, C., & Lillycrop, K. (2011). Child health, developmental plasticity, and epigenetic programming. *Endocrine reviews, 32*(2), 159-224.

Hoffmann, A., & Spengler, D. (2018). The mitochondrion as potential interface in early-life stress brain programming. *Frontiers in Behavioral Neuroscience, 12*, 306.

Holland, L. Z., Carvalho, J. E., Escriva, H., Laudet, V., Schubert, M., Shimeld, S. M., & Yu, J. (2013). Evolution of bilaterian central nervous systems: a single origin. *EvoDevo, 4*, 27.

Hoover, S. E. R., Winston, M. L., & Oldroyd, B. P. (2005, 2005/12/01). Retinue attraction and ovary activation: responses of wild type and anarchistic honey bees (Apis

mellifera) to queen and brood pheromones. *Behavioral Ecology and Sociobiology, 59*(2), 278-284. https://doi.org/10.1007/s00265-005-0039-2

Hopkins, A. (1917). A discussion of CG Hewitt's paper on "Insect Behaviour". *J. econ. Entomol, 10*, 92-93.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous Inference in General Parametric Models. *Biometrical Journal, 50*(3).

Hoverman, J. T., & Relyea, R. A. (2007). HOW FLEXIBLE IS PHENOTYPIC PLASTICITY? DEVELOPMENTAL WINDOWS FOR TRAIT INDUCTION AND REVERSAL. *Ecology, 88*(3), 693-705. https://doi.org/10.1890/05-1697

Huang, Z.-Y., & Otis, G. W. (1991). Inspection and feeding of larvae by worker honey bees (Hymenoptera: Apidae): effect of starvation and food quantity. *Journal of Insect Behavior, 4*(3), 305-317.

Hunt, G. (2007). Flight and fight: a comparative view of the neurophysiology and genetics of honey bee defensive behavior. *Journal of Insect Physiology, 53*(5), 399-410.

Hunt, G. J., Guzmán-Novoa, E., Uribe-Rubio, J. L., & Prieto-Merlos, D. (2003, 2003/09/01/). Genotype–environment interactions in honeybee guarding behaviour. *Animal Behaviour, 66*(3), 459-467. https://doi.org/https://doi.org/10.1006/anbe.2003.2253

Iba, M., Nagao, T., & Urano, A. (1995). Effects of population density on growth, behavior and levels of biogenic amines in the cricket, Gryllus bimaculatus. *Zoological science, 12*(6), 695-702.

Imdorf, A., Rickli, M., Kilchenmann, V., Bogdanov, S., & Wille, H. (1998). Nitrogen and mineral constituents of honey bee worker brood during pollen shortage. *Apidologie, 29*(4), 315-325.

Iovinella, I., Cappa, F., Cini, A., Petrocelli, I., Cervo, R., Turillazzi, S., & Dani, F. R. (2018). Antennal protein profile in honeybees: caste and task matter more than age. *Frontiers in Physiology, 9*, 748.

Ireland, T., & Garnier, S. (2018). Architecture, space and information in constructions built by humans and social insects: a conceptual review. *Philosophical Transactions of the Royal Society B: Biological Sciences, 373*(1753), 20170244.

Ironson, G., Field, T., Scafidi, F., Hashimoto, M., Kumar, M., Kumar, A., Price, A., Goncalves, A., Burman, I., Tetenman, C., Patarca, R., & Fletcher, M. A. (1996, 1996/01/01). Massage Therapy is Associated with Enhancement of the Immune System's Cytotoxic Capacity. *International Journal of Neuroscience, 84*(1-4), 205-217. https://doi.org/10.3109/00207459608987266

Ito, K., Shinomiya, K., Ito, M., Armstrong, J. D., Boyan, G., Hartenstein, V., Harzsch, S., Heisenberg, M., Homberg, U., Jenett, A., Keshishian, H., Restifo, Linda L., Rössler, W., Simpson, Julie H., Strausfeld, Nicholas J., Strauss, R., & Vosshall, Leslie B. (2014, 2014/02/19/). A Systematic Nomenclature for the Insect Brain. *Neuron, 81*(4), 755-765. https://doi.org/https://doi.org/10.1016/j.neuron.2013.12.017

Jaenisch, R., & Bird, A. (2003, 2003/03/01). Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nature Genetics, 33*(3), 245-254. https://doi.org/10.1038/ng1089

Jandt, J. M., Suryanarayanan, S., Hermanson, J. C., Jeanne, R. L., & Toth, A. L. (2017). Maternal and nourishment factors interact to influence offspring developmental trajectories in social wasps. *Proceedings of the Royal Society B: Biological Sciences, 284*(1857), 20170651. https://doi.org/doi:10.1098/rspb.2017.0651

Jay, S. (1964). Starvation studies of larval honey bees. *Canadian Journal of Zoology, 42*(3), 455-462.

Jenness, R. (1979). The composition of human milk. Seminars in perinatology,

Jeon, J. H., Moon, K., Kim, Y., & Kim, Y. H. (2020, 2020/08/18). Reference gene selection for qRT-PCR analysis of season- and tissue-specific gene expression profiles in the honey bee Apis mellifera. *Scientific Reports, 10*(1), 13935. https://doi.org/10.1038/s41598-020-70965-4

Jeong, H., Wu, X., Smith, B., & Yi, S. V. (2018). Genomic Landscape of Methylation Islands in Hymenopteran Insects. *Genome Biology and Evolution, 10*(10), 2766-2776. https://doi.org/10.1093/gbe/evy203

Jernigan, C. M., Halby, R., Gerkin, R. C., Sinakevitch, I., Locatelli, F., & Smith, B. H. (2020). Experience-dependent tuning of early olfactory processing in the adult honey bee, Apis mellifera. *The Journal of Experimental Biology, 223*(1), jeb206748. https://doi.org/10.1242/jeb.206748

Jobson, M. A., Jordan, J. M., Sandrof, M. A., Hibshman, J. D., Lennox, A. L., & Baugh, L. R. (2015). Transgenerational Effects of Early Life Starvation on Growth, Reproduction, and Stress Resistance in Caenorhabditis elegans. *Genetics, 201*(1), 201-212. https://doi.org/10.1534/genetics.115.178699

Joerges, J., Küttner, A., Galizia, C. G., & Menzel, R. (1997, 1997/05/01). Representations of odours and odour mixtures visualized in the honeybee brain. *Nature, 387*(6630), 285-288. https://doi.org/10.1038/387285a0

Johnson, B. R. (2008, 2008/03/01). Within-nest temporal polyethism in the honey bee. *Behavioral Ecology and Sociobiology, 62*(5), 777-784. https://doi.org/10.1007/s00265-007-0503-2

Kaftanoglu, O., Linksvayer, T. A., & Page, R. E., Jr. (2011). Rearing honey bees, Apis mellifera, in vitro I: Effects of sugar concentrations on survival and development. *Journal of Insect Science, 11*(1). https://doi.org/10.1673/031.011.9601

Kaissling, K.-E., & Priesner, E. (1970, 1970/01/01). Die Riechschwelle des Seidenspinners. *Naturwissenschaften, 57*(1), 23-28. https://doi.org/10.1007/BF00593550

Kamelska, A. M., Pietrzak-Fiećko, R., & Bryl, K. (2012). Variation of the cholesterol content in breast milk during 10 days collection at early stages of lactation. *Acta Biochimica Polonica, 59*(2).

Kano, F., & Call, J. (2014, 2014/05/01/). Cross-species variation in gaze following and conspecific preference among great apes, human infants and adults. *Animal Behaviour, 91*, 137-150. https://doi.org/https://doi.org/10.1016/j.anbehav.2014.03.011

Kapheim, K. M., Pan, H., Li, C., Salzberg, S. L., Puiu, D., Magoc, T., Robertson, H. M., Hudson, M. E., Venkat, A., & Fischman, B. J. (2015). Genomic signatures of evolutionary transitions from solitary to group living. *Science, 348*(6239), 1139-1143.

Kaplan, G. (2014). Animal communication. *Wiley Interdisciplinary Reviews: Cognitive Science, 5*(6), 661-677.

[Record #733 is using a reference type undefined in this output style.]

Kassambara, A. (2020). *ggpubr: 'ggplot2' Based Publication Ready Plots.* In https://CRAN.R-project.org/package=ggpubr

Kasumovic, M. M., & Brooks, R. C. (2011). It's All Who You Know: The Evolution Of Socially Cued Anticipatory Plasticity As A Mating Strategy. *The Quarterly Review of Biology, 86*(3), 181-197. https://doi.org/10.1086/661119

Kaupp, U. B. (2010, 2010/03/01). Olfactory signalling in vertebrates and insects: differences and commonalities. *Nature Reviews Neuroscience, 11*(3), 188-200. https://doi.org/10.1038/nrn2789

Kazlauskas, N., Klappenbach, M., Depino, A. M., & Locatelli, F. F. (2016). Sickness behavior in honey bees. *Frontiers in Physiology, 7*, 261.

Kelley, K. W., Bluthé, R.-M., Dantzer, R., Zhou, J.-H., Shen, W.-H., Johnson, R. W., & Broussard, S. R. (2003, 2003/02/15/). Cytokine-induced sickness behavior. *Brain, Behavior, and Immunity, 17*(1, Supplement), 112-118. https://doi.org/https://doi.org/10.1016/S0889-1591(02)00077-6

Kennedy, P. (2021). Haplodiploidy. In *Encyclopedia of social insects* (pp. 477-489). Springer.

Kenny, E., Birkhead, T. R., & Green, J. P. (2017). Allopreening in birds is associated with parental cooperation over offspring care and stable pair bonds across years. *Behavioral Ecology, 28*(4), 1142-1148. https://doi.org/10.1093/beheco/arx078

Khan, S., Prime, D. K., Hepworth, A. R., Lai, C. T., Trengove, N. J., & Hartmann, P. E. (2013). Investigation of Short-term Variations in Term Breast Milk Composition during Repeated Breast Expression Sessions. *Journal of Human Lactation, 29*(2), 196-204. https://doi.org/10.1177/0890334412470213

Kinoshita, M., & Homberg, U. (2017). Insect Brains: Minute Structures Controlling Complex Behaviors. In S. Shigeno, Y. Murakami, & T. Nomura (Eds.), *Brain Evolution by Design: From Neural Origin to Cognitive Architecture* (pp. 123-151). Springer Japan. https://doi.org/10.1007/978-4-431-56469-0_6

Klein, A.-M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences, 274*(1608), 303-313. https://doi.org/doi:10.1098/rspb.2006.3721

Klowden, M. J. (2013). *Physiological systems in insects*. Academic press.

Kluser, S., & Peduzzi, P. (2007). Global pollinator decline: a literature review. *Geneva: UNEP/GRID*.

Knecht, D., & Kaatz, H. (1990). Patterns of larval food production by hypopharyngeal glands in adult worker honey bees. *Apidologie, 21*(5), 457-468.

Koeniger, G. (1986). Reproduction and mating behavior. *Bee genetics and breeding*, 255-280.

Kohno, H., Suenami, S., Takeuchi, H., Sasaki, T., & Kubo, T. (2016). Production of knockout mutants by CRISPR/Cas9 in the European honeybee, Apis mellifera L. *Zoological science, 33*(5), 505-512.

König, K., Krimmer, E., Brose, S., Gantert, C., Buschlüter, I., König, C., Klopfstein, S., Wendt, I., Baur, H., & Krogmann, L. (2015). Does early learning drive ecological divergence during speciation processes in parasitoid wasps? *Proceedings of the Royal Society B: Biological Sciences, 282*(1799), 20141850.

Korosi, A., Naninck, E. F. G., Oomen, C. A., Schouten, M., Krugers, H., Fitzsimons, C., & Lucassen, P. J. (2012, 2012/02/14/). Early-life stress mediated modulation of adult neurogenesis and behavior. *Behavioural Brain Research, 227*(2), 400-409. https://doi.org/https://doi.org/10.1016/j.bbr.2011.07.037

Kovac, H., Stabentheiner, A., & Brodschneider, R. (2009). Contribution of honeybee drones of different age to colonial thermoregulation. *Apidologie, 40*(1), 82-95.

Kretzschmar, D., & Pflugfelder, G. O. (2002, 2002/01/01/). Glia in development, function, and neurodegeneration of the adult insect brain. *Brain Research Bulletin, 57*(1), 121-131. https://doi.org/https://doi.org/10.1016/S0361-9230(01)00643-8

Kucharski, R., Maleszka, J., Foret, S., & Maleszka, R. (2008, Mar 28). Nutritional control of reproductive status in honeybees via DNA methylation. *Science, 319*(5871), 1827-1830. https://doi.org/10.1126/science.1153069

Kunc, H. P., & Schmidt, R. (2019). The effects of anthropogenic noise on animals: a meta-analysis. *Biology Letters, 15*(11), 20190649.

Kundakovic, M., & Champagne, F. A. (2015, 2015/01/01). Early-Life Experience, Epigenetics, and the Developing Brain. *Neuropsychopharmacology, 40*(1), 141-153. https://doi.org/10.1038/npp.2014.140

Kuswadi, A. N. (1992). Allogrooming behavior of European honey bees Apis melliferal L.

Laben, R. C. (1963, 1963/11/01/). Factors Responsible for Variation in Milk Composition. *Journal of Dairy Science, 46*(11), 1293-1301. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(63)89264-4

Lan, J., Ding, G., Ma, W., Jiang, Y., & Huang, J. (2021). Pollen Source Affects Development and Behavioral Preferences in Honey Bees. *Insects, 12*(2), 130. https://www.mdpi.com/2075-4450/12/2/130

Land, B. B., & Seeley, T. D. (2004). The grooming invitation dance of the honey bee. *Ethology, 110*(1), 1-10.

Langer, J. (1929). Der Futtersaft, die Kost des Bienenkindes. *Bienen Vater, 61*, 25-30.

Larsen, A., Reynaldi, F. J., & Guzmán-Novoa, E. (2019). Fundaments of the honey bee (Apis mellifera) immune system. Review. *Revista mexicana de ciencias pecuarias, 10*(3), 705-728.

LeBoeuf, A. C., Benton, R., & Keller, L. (2013, 2013/02/01/). The molecular basis of social behavior: models, methods and advances. *Current Opinion in Neurobiology, 23*(1), 3-10. https://doi.org/https://doi.org/10.1016/j.conb.2012.08.008

Lemonnier, C., Bize, P., Boonstra, R., Dobson, F. S., Criscuolo, F., & Viblanc, V. A. (2022, 2022/09/01/). Effects of the social environment on vertebrate fitness and health in nature: Moving beyond the stress axis. *Hormones and Behavior, 145*, 105232. https://doi.org/https://doi.org/10.1016/j.yhbeh.2022.105232

Lenth, R. V. (2022). *emmeans: Estimated Marginal Means, aka Least-Squares Means.* . In

Letzkus, P., Ribi, W. A., Wood, J. T., Zhu, H., Zhang, S.-W., & Srinivasan, M. V. (2006, 2006/07/25/). Lateralization of Olfaction in the Honeybee Apis mellifera. *Current Biology, 16*(14), 1471-1476. https://doi.org/https://doi.org/10.1016/j.cub.2006.05.060

Lewis, R. J. (2022). Aggression, rank and power: why hens (and other animals) do not always peck according to their strength. *Philosophical Transactions of the Royal Society B, 377*(1845), 20200434.

Lhomme, P., Carrasco, D., Larsson, M., Hansson, B., & Anderson, P. (2017). A context-dependent induction of natal habitat preference in a generalist herbivorous insect. *Behavioral Ecology, 29*(2), 360-367. https://doi.org/10.1093/beheco/arx173

Li-Byarlay, H. (2016). The function of DNA methylation marks in social insects. *Frontiers in Ecology and Evolution, 4*, 57.

Li-Byarlay, H., Rittschof, C. C., Massey, J. H., Pittendrigh, B. R., & Robinson, G. E. (2014). Socially responsive effects of brain oxidative metabolism on aggression. *Proceedings of the National Academy of Sciences, 111*(34), 12533-12537.

Li, L., MaBouDi, H., Egertová, M., Elphick, M. R., Chittka, L., & Perry, C. J. (2017). A possible structural correlate of learning performance on a colour discrimination task in the brain of the bumblebee. *Proceedings of the Royal Society B: Biological Sciences, 284*(1864), 20171323.

Li, W., Sorensen, P. W., & Gallaher, D. D. (1995). The olfactory system of migratory adult sea lamprey (Petromyzon marinus) is specifically and acutely sensitive to unique bile acids released by conspecific larvae. *Journal of General Physiology, 105*(5), 569-587. https://doi.org/10.1085/jgp.105.5.569

Libbrecht, R., Oxley, P. R., Keller, L., & Kronauer, D. J. (2016, Feb 8). Robust DNA Methylation in the Clonal Raider Ant Brain. *Curr Biol, 26*(3), 391-395. https://doi.org/10.1016/j.cub.2015.12.040

Lichtneckert, R., & Reichert, H. (2005, 2005/05/01). Insights into the urbilaterian brain: conserved genetic patterning mechanisms in insect and vertebrate brain development. *Heredity, 94*(5), 465-477. https://doi.org/10.1038/sj.hdy.6800664

Lindauer, M., & Watkin, B. (1953). Division of labour in the honeybee colony. *Bee World, 34*(4), 63-73.

Linksvayer, T. A., Fewell, J. H., Gadau, J., & Laubichler, M. D. (2012). Developmental Evolution in Social Insects: Regulatory Networks from Genes to Societies. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution, 318*(3), 159-169. https://doi.org/10.1002/jez.b.22001

Liolios, V., Tananaki, C., Dimou, M., Kanelis, D., Goras, G., Karazafiris, E., & Thrasyvoulou, A. (2015, 2015/10/20). Ranking pollen from bee plants according to their protein contribution to honey bees. *Journal of Apicultural Research, 54*(5), 582-592. https://doi.org/10.1080/00218839.2016.1173353

Liu, Y., Henderson, G., Mao, L., & Laine, R. A. (2005). Effects of Temperature and Nutrition on Juvenile Hormone Titers of Coptotermes formosanus (Isoptera: Rhinotermitidae). *Annals of the Entomological Society of America, 98*(5), 732-737. https://doi.org/10.1603/0013-8746(2005)098[0732:Eotano]2.0.Co;2

Lnenicka, G., & Murphey, R. (1989). The refinement of invertebrate synapses during development. *Journal of Neurobiology, 20*(5), 339-355.

Lo, N., Simpson, S. J., & Sword, G. A. (2018). Epigenetics and developmental plasticity in orthopteroid insects. *Current Opinion in Insect Science, 25*, 25-34.

Locke, B. (2016, 2016/05/01). Natural Varroa mite-surviving Apis mellifera honeybee populations. *Apidologie, 47*(3), 467-482. https://doi.org/10.1007/s13592-015-0412-8

Loesel, R. (2011). Neurophylogeny: Retracing Early Metazoan Brain Evolution. In P. Pontarotti (Ed.), *Evolutionary Biology – Concepts, Biodiversity, Macroevolution and Genome Evolution* (pp. 169-191). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-20763-1_11

Lopes, P. C., French, S. S., Woodhams, D. C., & Binning, S. A. (2021). Sickness behaviors across vertebrate taxa: proximate and ultimate mechanisms. *Journal of Experimental Biology, 224*(9). https://doi.org/10.1242/jeb.225847

Losada-Perez, M. (2018, Mar - Jun). Glia: from 'just glue' to essential players in complex nervous systems: a comparative view from flies to mammals. *J Neurogenet, 32*(2), 78-91. https://doi.org/10.1080/01677063.2018.1464568

Lourenço, A. P., Mackert, A., dos Santos Cristino, A., & Simões, Z. L. P. (2008). Validation of reference genes for gene expression studies in the honey bee, Apis mellifera, by quantitative real-time RT-PCR. *Apidologie, 39*(3), 372-385.

Lowe, W. H., Martin, T. E., Skelly, D. K., & Woods, H. A. (2021, 2021/01/04/). Metamorphosis in an Era of Increasing Climate Variability. *Trends in Ecology & Evolution*. https://doi.org/https://doi.org/10.1016/j.tree.2020.11.012

Lucht-Bertram, E. (1961). DAS POSTEMBRYONALE WACHSTUM VON HIRNTEILEN BEI APIS MELLIFICA L. UND MYRMELEON EUROPAEUS L. *Zeitschrift für Morphologie und Ökologie der Tiere, 50*(5), 543-575. www.jstor.org/stable/43262092

Lüdecke et al. (2021). performance: An R Package for Assessment, Comparison and Testing of Statistical

Models. . *Journal of Open Source Software, 6*(60), 3139.

Łukasik, P. (2010). Trophic dimorphism in alternative male reproductive morphs of the acarid mite Sancassania berlesei. *Behavioral Ecology, 21*(2), 270-274. https://doi.org/10.1093/beheco/arp186

Luo, L., & O'Leary, D. D. (2005). Axon retraction and degeneration in development and disease. *Annu Rev Neurosci, 28*, 127-156. https://doi.org/10.1146/annurev.neuro.28.061604.135632

Maini, S., Medrzycki, P., & Porrini, C. (2010). The puzzle of honey bee losses: a brief review. *Bulletin of insectology, 63*(1), 153-160.

Maisonnasse, A., Lenoir, J.-C., Costagliola, G., Beslay, D., Choteau, F., Crauser, D., Becard, J.-M., Plettner, E., & Le Conte, Y. (2009). A scientific note on E-$\beta$-ocimene, a new volatile primer pheromone that inhibits worker ovary development in honey bees. *Apidologie, 40*(5), 562-564. https://doi.org/10.1051/apido/2009024

Malagnini, V., Cappellari, A., Marini, L., Zanotelli, L., Zorer, R., Angeli, G., Ioriatti, C., & Fontana, P. (2022). Seasonality and landscape composition drive the diversity of pollen collected by managed honey bees. *Frontiers in Sustainable Food Systems, 6*, 865368.

Manzi, C., Vergara-Amado, J., Franco, L. M., & Silva, A. X. (2020, 2020/10/01/). The effect of temperature on candidate gene expression in the brain of honey bee Apis mellifera (Hymenoptera: Apidae) workers exposed to neonicotinoid imidacloprid. *Journal of Thermal Biology, 93*, 102696. https://doi.org/https://doi.org/10.1016/j.jtherbio.2020.102696

Marfaing, P., Rouault, J., & Laffort, P. (1989, 1989/01/01/). Effect of the concentration and nature of olfactory stimuli on the proboscis extension of conditioned honey bees Apis mellifica ligustica. *Journal of Insect Physiology, 35*(12), 949-955. https://doi.org/https://doi.org/10.1016/0022-1910(89)90018-8

Marler, P., & Vandenbergh, J. G. (1979). *Social behavior and communication* (Vol. 3). Plenum Press.

Marshall, J. A. R., Bogacz, R., Dornhaus, A., Planqué, R., Kovacs, T., & Franks, N. R. (2009). On optimal decision-making in brains and social insect colonies. *Journal of The Royal Society Interface, 6*(40), 1065-1074. https://doi.org/doi:10.1098/rsif.2008.0511

Martin, S. J., & Medina, L. M. (2004). Africanized honeybees have unique tolerance to Varroa mites. *TRENDS in Parasitology, 20*(3), 112-114.

Mas, F., & Kölliker, M. (2008). Maternal care and offspring begging in social insects: chemical signalling, hormonal regulation and evolution. *Animal Behaviour, 76*(4), 1121-1131.

Matheson, M. D., & Bernstein, I. S. (2000, 2000/07/01). Grooming, social bonding, and agonistic aiding in rhesus monkeys [https://doi.org/10.1002/1098-2345(200007)51:3<177::AID-AJP2>3.0.CO;2-K]. *American Journal of Primatology, 51*(3), 177-186. https://doi.org/https://doi.org/10.1002/1098-2345(200007)51:3<177::AID-AJP2>3.0.CO;2-K

Mathot, K. J., Wright, J., Kempenaers, B., & Dingemanse, N. J. (2012). Adaptive strategies for managing uncertainty may explain personality-related differences in behavioural plasticity. *Oikos, 121*(7), 1009-1020.

Mattila, H. R., & Otis, G. W. (2006). The effects of pollen availability during larval development on the behaviour and physiology of spring-reared honey bee workers. *Apidologie, 37*(5), 533-546.

Mayseless, O., Berns, D. S., Yu, X. M., Riemensperger, T., Fiala, A., & Schuldiner, O. (2018, Sep 19). Developmental Coordination during Olfactory Circuit Remodeling in Drosophila. *Neuron, 99*(6), 1204-1215 e1205. https://doi.org/10.1016/j.neuron.2018.07.050

Mazerolle, M. J. (2020). *AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c)*. In

McDermott, J. H. (2009). The cocktail party problem. *Current Biology, 19*(22), R1024-R1027.

McFarland, R. Grooming. In *The International Encyclopedia of Biological Anthropology* (pp. 1-3). https://doi.org/https://doi.org/10.1002/9781118584538.ieba0217

McNamara, J. M., Trimmer, P. C., Eriksson, A., Marshall, J. A. R., & Houston, A. I. (2011). Environmental variability can select for optimism or pessimism. *Ecology Letters, 14*(1), 58-62. https://doi.org/https://doi.org/10.1111/j.1461-0248.2010.01556.x

Mello, T., Aleixo, A., Pinheiro, D., Nunes, F., Cristino, A., Bitondi, M., Barchuk, A., & Simões, Z. (2019). Hormonal control and target genes of ftz-f1 expression in the honeybee Apis mellifera: a positive loop linking juvenile hormone, ftz-f1, and vitellogenin. *Insect molecular biology, 28*(1), 145-159.

Michaelsen, K. F., Skafte, L., Badsberg, J. H., & Jørgensen, M. (1990). Variation in Macronutrients in Human Bank Milk: Influencing Factors and Implications for Human Milk Banking. *Journal of Pediatric Gastroenterology and Nutrition, 11*(2). https://journals.lww.com/jpgn/Fulltext/1990/08000/Variation_in_Macronutrients_in_Human_Bank_Milk_.13.aspx

Milinski, M. (1990). Information overload and food selection. In *Behavioural mechanisms of food selection* (pp. 721-737). Springer.

Miller, G. E., Chen, E., Fok, A. K., Walker, H., Lim, A., Nicholls, E. F., Cole, S., & Kobor, M. S. (2009). Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. *Proceedings of the National Academy of Sciences, 106*(34), 14716-14721.

Minoli, S., Kauer, I., Colson, V., Party, V., Renou, M., Anderson, P., Gadenne, C., Marion-Poll, F., & Anton, S. (2012). Brief Exposure to Sensory Cues Elicits Stimulus-Nonspecific General Sensitization in an Insect. *PLOS ONE, 7*(3), e34141. https://doi.org/10.1371/journal.pone.0034141

Miura, T. (2004, March 01). Proximate mechanisms and evolution of caste polyphenism in social insects: From sociality to genes [journal article]. *Ecological Research, 19*(2), 141-148. https://doi.org/10.1111/j.1440-1703.2003.00618.x

Moczek, A. P., & Emlen, D. J. (2000, 2000/02/01/). Male horn dimorphism in the scarab beetle, Onthophagus taurus: do alternative reproductive tactics favour alternative phenotypes? *Animal Behaviour, 59*(2), 459-466. https://doi.org/https://doi.org/10.1006/anbe.1999.1342

Mondet, F., Alaux, C., Severac, D., Rohmer, M., Mercer, A. R., & Le Conte, Y. (2015, 2015/05/22). Antennae hold a key to Varroa-sensitive hygiene behaviour in honey bees. *Scientific Reports, 5*(1), 10454. https://doi.org/10.1038/srep10454

Moore, A. J., Breed, M. D., & Moor, M. J. (1987). The guard honey bee: ontogeny and behavioural variability of workers performing a specialized task. *Animal Behaviour, 35*(4), 1159-1167.

Moorhouse, J. E., Fosbrooke, I. H., & Ludlow, A. R. (1987, 1987). Stopping a walking locust with sound: an analysis of variation in behavioural threshold. *Experimental biology, 46*(4), 193-201. http://europepmc.org/abstract/MED/3582590

Moray, N. (1959, 1959/02/01). Attention in dichotic listening: Affective cues and the influence of instructions. *Quarterly Journal of Experimental Psychology, 11*(1), 56-60. https://doi.org/10.1080/17470215908416289

Morhenn, V., Beavin, L. E., & Zak, P. J. (2012, Nov-Dec). Massage increases oxytocin and reduces adrenocorticotropin hormone in humans. *Altern Ther Health Med, 18*(6), 11-18.

Moritz, R., & Bürgin, H. (1987). Group response to alarm pheromones in social wasps and the honeybee. *Ethology, 76*(1), 15-26.

Morrison, I. (2016, 2016/12/01). Keep Calm and Cuddle on: Social Touch as a Stress Buffer. *Adaptive Human Behavior and Physiology, 2*(4), 344-362. https://doi.org/10.1007/s40750-016-0052-x

Müller, C. (2018, 2018/08/01/). Impacts of sublethal insecticide exposure on insects — Facts and knowledge gaps. *Basic and Applied Ecology, 30*, 1-10. https://doi.org/https://doi.org/10.1016/j.baae.2018.05.001

Müller, T., Küll, C. L., & Müller, C. (2016, 2016/12/01). Effects of larval versus adult density conditions on reproduction and behavior of a leaf beetle. *Behavioral Ecology and Sociobiology, 70*(12), 2081-2091. https://doi.org/10.1007/s00265-016-2212-1

Muscedere, M. L., & Traniello, J. F. (2012). Division of labor in the hyperdiverse ant genus Pheidole is associated with distinct subcaste-and age-related patterns of worker brain organization. *PLOS ONE, 7*(2), e31618.

Myllymäki, H., & Rämet, M. (2014). JAK/STAT Pathway in Drosophila Immunity. *Scandinavian Journal of Immunology, 79*(6), 377-385. https://doi.org/https://doi.org/10.1111/sji.12170

[Record #774 is using a reference type undefined in this output style.]

Nelson-Coffey, S. K., Fritz, M. M., Lyubomirsky, S., & Cole, S. W. (2017, 2017/07/01/). Kindness in the blood: A randomized controlled trial of the gene regulatory impact of prosocial behavior. *Psychoneuroendocrinology, 81*, 8-13. https://doi.org/https://doi.org/10.1016/j.psyneuen.2017.03.025

Nettle, D., & Bateson, M. (2015). Adaptive developmental plasticity: what is it, how can we recognize it and when can it evolve? *Proceedings of the Royal Society B: Biological Sciences, 282*(1812), 20151005.

Newsom, K. D., Moczek, A. P., & Schwab, D. B. (2019). Serotonin differentially affects morph-specific behavior in divergent populations of a horned beetle. *Behavioral Ecology*. https://doi.org/10.1093/beheco/arz192

Niven, J. E., & Laughlin, S. B. (2008). Energy limitation as a selective pressure on the evolution of sensory systems. *Journal of Experimental Biology, 211*(11), 1792-1804.

Noël, A., Dumas, C., Rottier, E., Beslay, D., Costagliola, G., Ginies, C., Nicolè, F., Rau, A., Le Conte, Y., & Mondet, F. (2023). Detailed chemical analysis of honey bee (Apis mellifera) worker brood volatile profile from egg to emergence. *PLOS ONE, 18*(2), e0282120. https://doi.org/10.1371/journal.pone.0282120

Noël, A., Le Conte, Y., & Mondet, F. (2020). Varroa destructor: how does it harm Apis mellifera honey bees and what can be done about it? *Emerging Topics in Life Sciences, 4*(1), 45-57. https://doi.org/10.1042/etls20190125

Nothling, J., Malan-Mueller, S., Abrahams, N., Hemmings, S. M. J., & Seedat, S. (2019). Epigenetic alterations associated with childhood trauma and adult mental health outcomes: a systematic review. *The World Journal of Biological Psychiatry, 21*(7). https://doi.org/10.1080/15622975.2019.1583369

Nunn, C., Altizer, S., & Altizer, S. M. (2006). *Infectious diseases in primates: behavior, ecology and evolution*. Oxford University Press.

Nunn, C. L., Jordán, F., McCabe, C. M., Verdolin, J. L., & Fewell, J. H. (2015). Infectious disease and group size: more than just a numbers game. *Philosophical Transactions of the Royal Society B: Biological Sciences, 370*(1669), 20140111. https://doi.org/doi:10.1098/rstb.2014.0111

Nuotclà, J. A., Biedermann, P. H. W., & Taborsky, M. (2019). Pathogen defence is a potential driver of social evolution in ambrosia beetles. *Proceedings of the Royal Society B: Biological Sciences, 286*(1917), 20192332. https://doi.org/doi:10.1098/rspb.2019.2332

[Record #775 is using a reference type undefined in this output style.]

O'Connell, L. A. (2013, 2013/09/01). Evolutionary Development of Neural Systems in Vertebrates and Beyond. *Journal of Neurogenetics, 27*(3), 69-85. https://doi.org/10.3109/01677063.2013.789511

O'Donnell, S., & Bulova, S. J. (2007, 2007/08/01). Worker connectivity: a review of the design of worker communication systems and their effects on task performance in insect societies. *Insectes Sociaux, 54*(3), 203-210. https://doi.org/10.1007/s00040-007-0945-6

Ochieng, S., Park, K., & Baker, T. (2002, 2002/05/01). Host plant volatiles synergize responses of sex pheromone-specific olfactory receptor neurons in male Helicoverpa zea. *Journal of Comparative Physiology A, 188*(4), 325-333. https://doi.org/10.1007/s00359-002-0308-8

Odeon, M. M., Salatino, A. E., & Acosta, G. B. (2013). Consequences of postnatal stress: maternal separation in rats induces long-lasting changes on glutamate transporters.

Odoux, J.-F., Feuillet, D., Aupinel, P., Loublier, Y., Tasei, J.-N., & Mateescu, C. (2012, 2012/09/01). Territorial biodiversity and consequences on physico-chemical characteristics of pollen collected by honey bee colonies. *Apidologie, 43*(5), 561-575. https://doi.org/10.1007/s13592-012-0125-1

Oftedal, O. T. (2012). The evolution of milk secretion and its ancient origins. *animal, 6*(3), 355-368. https://doi.org/10.1017/S1751731111001935

Oka, Y., Omura, M., Kataoka, H., & Touhara, K. (2004). Olfactory receptor antagonism between odorants. *The EMBO Journal, 23*(1), 120-126. https://doi.org/https://doi.org/10.1038/sj.emboj.7600032

Olvido, A. E., & Mousseau, T. A. (1995). Effect of rearing environment on calling-song plasticity in the striped ground cricket. *Evolution, 49*(6), 1271-1277.

Omar, E., Abd-Ella, A. A., Khodairy, M. M., Moosbeckhofer, R., Crailsheim, K., & Brodschneider, R. (2017). Influence of different pollen diets on the development of hypopharyngeal glands and size of acid gland sacs in caged honey bees (Apis mellifera). *Apidologie, 48*, 425-436.

Ord, T. J., Peters, R. A., Clucas, B., & Stamps, J. A. (2007). Lizards speed up visual displays in noisy motion habitats. *Proceedings of the Royal Society B: Biological Sciences, 274*(1613), 1057-1062.

Osborne, R. H. (1996, 1996/01/01/). Insect neurotransmission: Neurotransmitters and their receptors. *Pharmacology & Therapeutics, 69*(2), 117-142. https://doi.org/https://doi.org/10.1016/0163-7258(95)02054-3

Oster, G. F., & Wilson, E. O. (1978). *Caste and ecology in the social insects*. Princeton University Press.

Ott, S. R., & Rogers, S. M. (2010). Gregarious desert locusts have substantially larger brains with altered proportions compared with the solitarious phase. *Proceedings of the Royal Society B: Biological Sciences, 277*(1697), 3087-3096. https://doi.org/doi:10.1098/rspb.2010.0694

Page, R. E., & Peng, C. Y. S. (2001, 2001/04/01/). Aging and development in social insects with emphasis on the honey bee, Apis mellifera L. *Experimental Gerontology, 36*(4), 695-711. https://doi.org/https://doi.org/10.1016/S0531-5565(00)00236-9

Page, R. E., & Robinson, G. E. (1991). The genetics of division of labour in honey bee colonies. In *Advances in insect physiology* (Vol. 23, pp. 117-169). Elsevier.

Pahl, M., Si, A., & Zhang, S. (2013). Numerical cognition in bees and other insects. *Frontiers in psychology, 4*, 162.

Pankiw, T., Page Jr, R. E., & Kim Fondrk, M. (1998, 1998/12/01). Brood pheromone stimulates pollen foraging in honey bees (Apis mellifera). *Behavioral Ecology and Sociobiology, 44*(3), 193-198. https://doi.org/10.1007/s002650050531

Paredes, U., Radersma, R., Cannell, N., While, G. M., & Uller, T. (2016). Low incubation temperature induces DNA hypomethylation in lizard brains. *Journal of*

*Experimental Zoology Part A: Ecological Genetics and Physiology, 325*(6), 390-395.

Patalano, S., Hore, T. A., Reik, W., & Sumner, S. (2012, 2012/06/01/). Shifting behaviour: epigenetic reprogramming in eusocial insects. *Current Opinion in Cell Biology, 24*(3), 367-373. https://doi.org/https://doi.org/10.1016/j.ceb.2012.02.005

Patel, M. D. (1936). The Physiology of the Formation of" pigeon's Milk". *Physiological Zoology, 9*(2), 129-152.

Pattamayutanon, P., Peng, C.-C., Sinpoo, C., & Chantawannakul, P. (2018). Effects of pollen feeding on quality of royal jelly. *Journal of economic entomology, 111*(6), 2974-2978.

Paulino Simões, Z., L., Boleli, I., C., & Hartfelder, K. (1997). Occurrence of a prothoracicotropic hormone-like peptide in the developing nervous system of the honey bee (Apis mellifera L). *Apidologie, 28*(6), 399-409. https://doi.org/10.1051/apido:19970607

Pearce, A. N., Huang, Z. Y., & Breed, M. D. (2001, 2001/11/01/). Juvenile hormone and aggression in honey bees. *Journal of Insect Physiology, 47*(11), 1243-1247. https://doi.org/https://doi.org/10.1016/S0022-1910(01)00109-3

Pearson, K. (1993). Common principles of motor control in vertebrates and invertebrates. *Annual Review of Neuroscience, 16*(1), 265-297.

Peng, W., Ma, N. L., Zhang, D., Zhou, Q., Yue, X., Khoo, S. C., Yang, H., Guan, R., Chen, H., Zhang, X., Wang, Y., Wei, Z., Suo, C., Peng, Y., Yang, Y., Lam, S. S., & Sonne, C. (2020, 2020/12/01/). A review of historical and recent locust outbreaks: Links to global warming, food security and mitigation strategies. *Environmental Research, 191*, 110046. https://doi.org/https://doi.org/10.1016/j.envres.2020.110046

Peters, A., Schweiger, U., Pellerin, L., Hubold, C., Oltmanns, K., Conrad, M., Schultes, B., Born, J., & Fehm, H. (2004). The selfish brain: competition for energy resources. *Neuroscience & Biobehavioral Reviews, 28*(2), 143-180.

Petit, C., Le Ru, B., Dupas, S., Frérot, B., Ahuya, P., Kaiser-Arnauld, L., Harry, M., & Calatayud, P.-A. (2015). Influence of Dietary Experience on the Induction of Preference of Adult Moths and Larvae for a New Olfactory Cue. *PLOS ONE, 10*(8), e0136169-e0136169. https://doi.org/10.1371/journal.pone.0136169

Pettis, J. S., & Delaplane, K. S. (2010). Coordinated responses to honey bee decline in the USA. *Apidologie, 41*(3), 256-263.

Pilz, P. K. D., & Schnitzler, H.-U. (1996, 1996/07/01/). Habituation and Sensitization of the Acoustic Startle Response in Rats: Amplitude, Threshold, and Latency Measures. *Neurobiology of Learning and Memory, 66*(1), 67-79. https://doi.org/https://doi.org/10.1006/nlme.1996.0044

Preston, S. R., Palmer, J. H., Harrison, J. W., Carr, H. M., & Rittschof, C. C. (2019, 2019/10/01). The impacts of maternal stress on worker phenotypes in the honey bee. *Apidologie, 50*(5), 704-719. https://doi.org/10.1007/s13592-019-00680-1

Prieto Peres, M. F., & Valença, M. M. (2010). Chapter 60 - Headache: endocrinological aspects. In M. J. Aminoff, F. Boller, & D. F. Swaab (Eds.), *Handbook of Clinical Neurology* (Vol. 97, pp. 717-737). Elsevier. https://doi.org/https://doi.org/10.1016/S0072-9752(10)97060-7

Prillinger, L. (1981, 1981/03/01). Postembryonic development of the antennal lobes in Periplaneta americana L. *Cell and Tissue Research, 215*(3), 563-575. https://doi.org/10.1007/BF00233532

Pringle, J. (1940). The reflex mechanism of the insect leg. *Journal of Experimental Biology, 17*(1), 8-17.

Pritchard, D. J. (2016). Grooming by honey bees as a component of varroa resistant behavior. *Journal of Apicultural Research, 55*(1), 38-48.

Prudic, K. L., Jeon, C., Cao, H., & Monteiro, A. (2011, Jan 7). Developmental plasticity in sexual roles of butterfly species drives mutual sexual ornamentation. *Science, 331*(6013), 73-75. https://doi.org/10.1126/science.1197114

Qin, Y., Shi, W., Zhuang, J., Liu, Y., Tang, L., Bu, J., Sun, J., & Bei, F. (2019). Variations in melatonin levels in preterm and term human breast milk during the first month after delivery. *Scientific Reports, 9*(1), 1-5.

Quan, N., & Banks, W. A. (2007, 2007/08/01/). Brain-immune communication pathways. *Brain, Behavior, and Immunity, 21*(6), 727-735. https://doi.org/https://doi.org/10.1016/j.bbi.2007.05.005

Quque, M., Ferreira, C., Sosa, S., Schull, Q., Zahn, S., Criscuolo, F., Bleu, J., & Viblanc, V. A. (2022). Cascading Effects of Conspecific Aggression on Oxidative Status and

Telomere Length in Zebra Finches. *Physiological and Biochemical Zoology, 95*(5), 416-429. https://doi.org/10.1086/721252

R Core Team. (2021). *R: A language and environment for statistical computing. R Foundation for Statistical Computing.* In

Rachinsky, A. (1994, 1994/07/01/). Octopamine and serotonin influence on corpora allata activity in honey bee (Apis mellifera) larvae. *Journal of Insect Physiology, 40*(7), 549-554. https://doi.org/https://doi.org/10.1016/0022-1910(94)90141-4

Radwan, J. (1995, 1995/06/01). Male morph determination in two species of acarid mites. *Heredity, 74*(6), 669-673. https://doi.org/10.1038/hdy.1995.91

Rajakumar, R., Koch, S., Couture, M., Fave, M. J., Lillico-Ouachour, A., Chen, T., De Blasis, G., Rajakumar, A., Ouellette, D., & Abouheif, E. (2018, Oct). Social regulation of a rudimentary organ generates complex worker-caste systems in ants. *Nature, 562*(7728), 574-577. https://doi.org/10.1038/s41586-018-0613-1

Ramanathan, A. N. K. G., Nair, A. J., & Sugunan, V. S. (2018). A review on Royal Jelly proteins and peptides. *Journal of Functional Foods, 44*, 255-264.

Randt, C. T., Blizard, D. A., & Friedman, E. (1975). Early life undernutrition and aggression in two mouse strains. *Developmental Psychobiology, 8*(3), 275-279. https://doi.org/https://doi.org/10.1002/dev.420080313

Ravary, F., Lecoutey, E., Kaminski, G., Châline, N., & Jaisson, P. (2007). Individual experience alone can generate lasting division of labor in ants. *Current Biology, 17*(15), 1308-1312.

Reh, R. K., Dias, B. G., Nelson, C. A., 3rd, Kaufer, D., Werker, J. F., Kolb, B., Levine, J. D., & Hensch, T. K. (2020, Sep 22). Critical period regulation across multiple timescales. *Proc Natl Acad Sci U S A, 117*(38), 23242-23251. https://doi.org/10.1073/pnas.1820836117

Reh, T., & Cagan, R. (1994). Intrinsic and extrinsic signals in the developing vertebrate and fly eyes: viewing vertebrate and invertebrate eyes in the same light. *Perspectives on developmental neurobiology, 2*(2), 183-190.

Reichert, H. (2009). Evolutionary conservation of mechanisms for neural regionalization, proliferation and interconnection in brain development. *Biology Letters, 5*(1), 112-116. https://doi.org/doi:10.1098/rsbl.2008.0337

Reilly, J., Artz, D., Biddinger, D., Bobiwash, K., Boyle, N., Brittain, C., Brokaw, J., Campbell, J., Daniels, J., & Elle, E. (2020). Crop production in the USA is frequently limited by a lack of pollinators. *Proceedings of the Royal Society B, 287*(1931), 20200922.

Reingold, S. C., & Camhi, J. M. (1977, 1977/01/01/). A quantitative analysis of rhythmic leg movements during three different behaviors in the cockroach, Periplaneta americana. *Journal of Insect Physiology, 23*(11), 1407-1420. https://doi.org/https://doi.org/10.1016/0022-1910(77)90166-4

Reinhard, J., & Srinivasan, M. V. (2009). The role of scents in honey bee foraging and recruitment. *Food exploitation by social insects: ecological, behavioral, and theoretical approaches, 1*, 165-182.

Rembold, H., Kremer, J.-P., & Ulrich, G. M. (1980). Characterization of postembryonic developmental stages of the female castes of the honey bee, Apis mellifera L. *Apidologie, 11*(1), 29-38.

Rembold, H., Lackner, B., & Geistbeck, I. (1974, 1974/02/01/). The chemical basis of honeybee, Apis mellifera, caste formation. Partial purification of queen bee determinator from royal jelly. *Journal of Insect Physiology, 20*(2), 307-314. https://doi.org/https://doi.org/10.1016/0022-1910(74)90063-8

Renzi, M. T., Rodríguez-Gasol, N., Medrzycki, P., Porrini, C., Martini, A., Burgio, G., Maini, S., & Sgolastra, F. (2016, 2016/11/01). Combined effect of pollen quality and thiamethoxam on hypopharyngeal gland development and protein content in Apis mellifera. *Apidologie, 47*(6), 779-788. https://doi.org/10.1007/s13592-016-0435-9

Richard, F. J., Aubert, A., & Grozinger, C. M. (2008, 2008/11/17). Modulation of social interactions by immune stimulation in honey bee, Apis mellifera, workers. *BMC Biology, 6*(1), 50. https://doi.org/10.1186/1741-7007-6-50

Riessberger, U., & Crailsheim, K. (1997). Short-term effect of different weather conditions upon the behaviour of forager and nurse honey bees (Apis mellifera carnica Pollmann). *Apidologie, 28*(6), 411-426.

Rietdorf, K., & Steidle, J. L. (2002). Was Hopkins right? Influence of larval and early adult experience on the olfactory response in the granary weevil Sitophilus granarius (Coleoptera, Curculionidae). *Physiological Entomology, 27*(3), 223-227.

Rillich, J., Rillich, B., & Stevenson, P. A. (2018). Differential modulation of courtship behavior and subsequent aggression by octopamine, dopamine and serotonin in male crickets. *Hormones and Behavior, 114*, 104542.

Rittschof, C. C. (2017, 2017/03/03). Sequential social experiences interact to modulate aggression but not brain gene expression in the honey bee (Apis mellifera). *Frontiers in Zoology, 14*(1), 16. https://doi.org/10.1186/s12983-017-0199-8

Rittschof, C. C., Bukhari, S. A., Sloofman, L. G., Troy, J. M., Caetano-Anolles, D., Cash-Ahmed, A., Kent, M., Lu, X., Sanogo, Y. O., Weisner, P. A., Zhang, H., Bell, A. M., Ma, J., Sinha, S., Robinson, G. E., & Stubbs, L. (2014, Dec 16). Neuromolecular responses to social challenge: common mechanisms across mouse, stickleback fish, and honey bee. *Proc Natl Acad Sci U S A, 111*(50), 17929-17934. https://doi.org/10.1073/pnas.1420369111

Rittschof, C. C., Coombs, C. B., Frazier, M., Grozinger, C. M., & Robinson, G. E. (2015, 10/23/online). Early-life experience affects honey bee aggression and resilience to immune challenge [Article]. *Scientific Reports, 5*, 15572. https://doi.org/10.1038/srep15572

https://www.nature.com/articles/srep15572#supplementary-information

Rittschof, C. C., & Robinson, G. E. (2013). Manipulation of colony environment modulates honey bee aggression and brain gene expression. *Genes, Brain and Behavior, 12*(8), 802-811.

Rittschof, C. C., Rubin, B. E. R., & Palmer, J. H. (2019, 2019/12/30). The transcriptomic signature of low aggression in honey bees resembles a response to infection. *BMC Genomics, 20*(1), 1029. https://doi.org/10.1186/s12864-019-6417-3

Rittschof, C. C., & Schirmeier, S. (2017). Insect models of central nervous system energy metabolism and its links to behavior. *Glia, 66*(6), 1160-1175. https://doi.org/10.1002/glia.23235

Rittschof, C. C., Vekaria, H. J., Palmer, J. H., & Sullivan, P. G. (2018). Brain mitochondrial bioenergetics change with rapid and prolonged shifts in aggression in the honey bee, Apis mellifera. *Journal of Experimental Biology, 221*(8), jeb176917.

Robertson, R. M., Dawson-Scully, K. D., & Andrew, R. D. (2020, Mar 1). Neural shutdown under stress: an evolutionary perspective on spreading depolarization. *J Neurophysiol, 123*(3), 885-895. https://doi.org/10.1152/jn.00724.2019

Robinson, G. E. (1985, 1985/01/01/). Effects of a juvenile hormone analogue on honey bee foraging behaviour and alarm pheromone production. *Journal of Insect Physiology, 31*(4), 277-282. https://doi.org/https://doi.org/10.1016/0022-1910(85)90003-4

Robinson, G. E. (1987a, 1987/09/01). Modulation of alarm pheromone perception in the honey bee: evidence for division of labor based on hormonall regulated response thresholds. *Journal of Comparative Physiology A, 160*(5), 613-619. https://doi.org/10.1007/BF00611934

Robinson, G. E. (1987b, 1987/05/01). Regulation of honey bee age polyethism by juvenile hormone. *Behavioral Ecology and Sociobiology, 20*(5), 329-338. https://doi.org/10.1007/BF00300679

Robinson, G. E., Page Jr, R. E., Strambi, C., & Strambi, A. (1992). Colony integration in honey bees: mechanisms of behavioral reversion. *Ethology, 90*(4), 336-348.

Roeder, T. (2005). TYRAMINE AND OCTOPAMINE: Ruling Behavior and Metabolism. *Annual Review of Entomology, 50*(1), 447-477. https://doi.org/10.1146/annurev.ento.50.071803.130404

Rolff, J., Johnston, P. R., & Reynolds, S. (2019). Complete metamorphosis of insects. *Philosophical Transactions of the Royal Society B, 374*(1783), 20190063.

Rolls, M. M., & Jegla, T. J. (2015). Neuronal polarity: an evolutionary perspective. *The Journal of Experimental Biology, 218*(4), 572-580. https://doi.org/10.1242/jeb.112359

Ronald, K. L., Fernández-Juricic, E., & Lucas, J. R. (2012, 2012/12/01/). Taking the sensory approach: how individual differences in sensory perception can influence mate choice. *Animal Behaviour, 84*(6), 1283-1294. https://doi.org/https://doi.org/10.1016/j.anbehav.2012.09.015

Rosa, P., & Koper, N. (2018). Integrating multiple disciplines to understand effects of anthropogenic noise on animal communication. *Ecosphere, 9*(2), e02127.

Rospars, J. P. (1988, 1988/01/01/). Structure and development of the insect antennodeutocerebral system. *International Journal of Insect Morphology and Embryology, 17*(3), 243-294. https://doi.org/https://doi.org/10.1016/0020-7322(88)90041-4

Rösvik, A., Lhomme, P., Khallaf, M. A., & Anderson, P. (2020). Plant-induced transgenerational plasticity affecting performance but not preference in a polyphagous moth. *Frontiers in Ecology and Evolution, 8*, 254.

Rubow, J., Cherry, M. I., & Sharpe, L. L. (2017, 2017/05/01/). Calling for help: dwarf mongoose recruitment calls inform receivers about context and elicit disparate responses. *Animal Behaviour, 127*, 7-14. https://doi.org/https://doi.org/10.1016/j.anbehav.2017.02.018

Russell, Y. I., & Phelps, S. (2013). How do you measure pleasure? A discussion about intrinsic costs and benefits in primate allogrooming. *Biology & Philosophy, 28*(6), 1005-1020.

Russo, J. M., & Ison, J. R. (1979, 1979/03/01). Sensitization of the rat's acoustic startle response by repetition of a photic stimulus. *Physiological Psychology, 7*(1), 102-106. https://doi.org/10.3758/BF03326627

Sakurai, T., Namiki, S., & Kanzaki, R. (2014). Molecular and neural mechanisms of sex pheromone reception and processing in the silkmoth Bombyx mori. *Frontiers in Physiology, 5*, 125.

Saleh, A., Potter, G. G., McQuoid, D. R., Boyd, B., Turner, R., MacFall, J. R., & Taylor, W. D. (2017, Jan). Effects of early life stress on depression, cognitive performance and brain morphology. *Psychol Med, 47*(1), 171-181. https://doi.org/10.1017/S0033291716002403

Salzberg, A., & Bellen, H. J. (1996). Invertebrate versus vertebrate neurogenesis: variations on the same theme? *Developmental genetics, 18*(1), 1-10.

Sato, S., Tarumizu, K., & Hatae, K. (1993, 1993/12/01/). The influence of social factors on allogrooming in cows. *Applied Animal Behaviour Science, 38*(3), 235-244. https://doi.org/https://doi.org/10.1016/0168-1591(93)90022-H

Saul, M. C., Blatti, C., Yang, W., Bukhari, S. A., Shpigler, H. Y., Troy, J. M., Seward, C. H., Sloofman, L., Chandrasekaran, S., Bell, A. M., Stubbs, L., Robinson, G. E., Zhao, S. D., & Sinha, S. (2019, Jan). Cross-species systems analysis of evolutionary toolkits of neurogenomic response to social challenge. *Genes Brain Behav, 18*(1), e12502. https://doi.org/10.1111/gbb.12502

Schaefer, P. L., & Ritzmann, R. E. (2001). Descending influences on escape behavior and motor pattern in the cockroach. *Journal of Neurobiology, 49*(1), 9-28. https://doi.org/10.1002/neu.1062

Scheiner, R., & Erber, J. (2009). Sensory thresholds, learning and the division of foraging labor in the honey bee. *Organization of insect societies: from genomes to socio-complexity. Harvard University Press, Cambridge*, 335-356.

Schilcher, F., Hilsmann, L., Ankenbrand, M. J., Krischke, M., Mueller, M. J., Steffan-Dewenter, I., & Scheiner, R. (2022, 2022-November-18). Honeybees are buffered against undernourishment during larval stages [Original Research]. *Frontiers in Insect Science, 2*. https://doi.org/10.3389/finsc.2022.951317

Schilcher, F., Hilsmann, L., Rauscher, L., Değirmenci, L., Krischke, M., Krischke, B., Ankenbrand, M., Rutschmann, B., Mueller, M. J., Steffan-Dewenter, I., & Scheiner, R. (2022). In Vitro Rearing Changes Social Task Performance and Physiology in Honeybees. *Insects, 13*(1), 4. https://www.mdpi.com/2075-4450/13/1/4

Schloerke, B., Cook, D., Larmarange, J., Briatte, F., Marbach, M., Thoen, E., Elberg, A., & Crowley, J. (2021). *GGally: Extension to 'ggplot2'*. In (Version 2.1.2.) https://CRAN.R-project.org/package=GGally

Schmickl, T., Blaschon, B., Gurmann, B., & Crailsheim, K. (2003). Collective and individual nursing investment in the queen and in young and old honeybee larvae during foraging and non-foraging periods. *Insectes Sociaux, 50*(2), 174-184.

Schmickl, T., & Crailsheim, K. (2001). Cannibalism and early capping: strategy of honeybee colonies in times of experimental pollen shortages. *Journal of Comparative Physiology, 187*(7), 541-547.

Schmickl, T., & Crailsheim, K. (2002, 2002/04/01). How honeybees (Apis mellifera L.) change their broodcare behaviour in response to non-foraging conditions and poor pollen conditions. *Behavioral Ecology and Sociobiology, 51*(5), 415-425. https://doi.org/10.1007/s00265-002-0457-3

Schmitz, R. J., Lewis, Z. A., & Goll, M. G. (2019, 2019/11/01/). DNA Methylation: Shared and Divergent Features across Eukaryotes. *Trends in Genetics, 35*(11), 818-827. https://doi.org/https://doi.org/10.1016/j.tig.2019.07.007

Schneider, S. S., & Lewis, L. A. (2004). The vibration signal, modulatory communication and the organization of labor in honey bees, Apis mellifera. *Apidologie, 35*(2), 117-131.

Schneider, S. S., & McNally, L. C. (1992). Colony Defense in the African Honey Bee in Africa (Hymenoptera: Apidae). *Environmental Entomology, 21*(6), 1362-1370. https://doi.org/10.1093/ee/21.6.1362

Schoofs, L., De Loof, A., & Van Hiel, M. B. (2017). Neuropeptides as Regulators of Behavior in Insects. *Annual Review of Entomology, 62*(1), 35-52. https://doi.org/10.1146/annurev-ento-031616-035500

Schowalter, T. D. (2016). *Insect ecology: an ecosystem approach*. Academic press.

Schretter, C. E., Vielmetter, J., Bartos, I., Marka, Z., Marka, S., Argade, S., & Mazmanian, S. K. (2018, Nov). A gut microbial factor modulates locomotor behaviour in Drosophila. *Nature, 563*(7731), 402-406. https://doi.org/10.1038/s41586-018-0634-9

Schröder, R., & Hilker, M. (2008). The Relevance of Background Odor in Resource Location by Insects: A Behavioral Approach. *BioScience, 58*(4), 308-316. https://doi.org/10.1641/b580406

Schwab, D. B., Casasa, S., & Moczek, A. P. (2018). On the Reciprocally Causal and Constructive Nature of Developmental Plasticity and Robustness. *Front Genet, 9*, 735. https://doi.org/10.3389/fgene.2018.00735

Schwander, T., Lo, N., Beekman, M., Oldroyd, B. P., & Keller, L. (2010, 2010/05/01/). Nature versus nurture in social insect caste differentiation. *Trends in Ecology & Evolution, 25*(5), 275-282. https://doi.org/https://doi.org/10.1016/j.tree.2009.12.001

Schwander, T., Rosset, H., & Chapuisat, M. (2005, 2005/12/01). Division of labour and worker size polymorphism in ant colonies: the impact of social and genetic factors. *Behavioral Ecology and Sociobiology, 59*(2), 215-221. https://doi.org/10.1007/s00265-005-0027-6

Scofield, H. N., & Mattila, H. R. (2015). Honey bee workers that are pollen stressed as larvae become poor foragers and waggle dancers as adults. *PLOS ONE, 10*(4), e0121731-e0121731. https://doi.org/10.1371/journal.pone.0121731

Scott, J. P. (1962). Critical periods in behavioral development. *Science, 138*(3544), 949-958.

Seeley, T. D. (1982, 1982/12/01). Adaptive significance of the age polyethism schedule in honeybee colonies. *Behavioral Ecology and Sociobiology, 11*(4), 287-293. https://doi.org/10.1007/BF00299306

Seeley, T. D. (1989). The Honey Bee Colony as a Superorganism. *American Scientist, 77*(6), 546-553. http://www.jstor.org/stable/27856005

Seeley, T. D. (1994). Honey bee foragers as sensory units of their colonies. *Behavioral Ecology and Sociobiology, 34*, 51-62.

Seeley, T. D. (1995). *The wisdom of the hive: the social physiology of honey bee colonies*. Harvard University Press.

Sehonova, P., Svobodova, Z., Dolezelova, P., Vosmerova, P., & Faggio, C. (2018, 2018/08/01/). Effects of waterborne antidepressants on non-target animals living in the aquatic environment: A review. *Science of The Total Environment, 631-632*, 789-794. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.03.076

Seltmann, M. W., Öst, M., Jaatinen, K., Atkinson, S., Mashburn, K., & Hollmén, T. (2012, 2012/10/01/). Stress responsiveness, age and body condition interactively affect flight initiation distance in breeding female eiders. *Animal Behaviour, 84*(4), 889-896. https://doi.org/https://doi.org/10.1016/j.anbehav.2012.07.012

Seyfarth, R. M., & Cheney, D. L. (2003). Signalers and Receivers in Animal Communication. *Annual Review of Psychology, 54*(1), 145-173. https://doi.org/10.1146/annurev.psych.54.101601.145121

Shen, F., Zhang, Z., Fu, Y., Zhang, Z., Sun, X., Dong, J., Ding, X., Chen, M., & Zhang, X. (2021). Effects of Food Deprivation Duration on the Behavior and Metabolism of Black Rockfish (Sebastes schlegelii). *Fishes, 6*(4), 58. https://www.mdpi.com/2410-3888/6/4/58

Shi, Y.-B. (2013). *Animal metamorphosis*. Academic Press.

Shi, Y. Y., Huang, Z. Y., Zeng, Z. J., Wang, Z. L., Wu, X. B., & Yan, W. Y. (2011). Diet and cell size both affect queen-worker differentiation through DNA methylation in honey bees (Apis mellifera, Apidae). *PLOS ONE, 6*(4), e18808-e18808. https://doi.org/10.1371/journal.pone.0018808

Shpigler, H. Y., Saul, M. C., Corona, F., Block, L., Cash Ahmed, A., Zhao, S. D., & Robinson, G. E. (2017). Deep evolutionary conservation of autism-related genes. *Proceedings of the National Academy of Sciences, 114*(36), 9653-9658. https://doi.org/10.1073/pnas.1708127114

Shpigler, H. Y., Saul, M. C., Murdoch, E. E., Cash-Ahmed, A. C., Seward, C. H., Sloofman, L., Chandrasekaran, S., Sinha, S., Stubbs, L. J., & Robinson, G. E. (2017). Behavioral, transcriptomic and epigenetic responses to social challenge in honey bees. *Genes, Brain and Behavior, 16*(6), 579-591. https://doi.org/https://doi.org/10.1111/gbb.12379

Shpigler, H. Y., Saul, M. C., Murdoch, E. E., Corona, F., Cash-Ahmed, A. C., Seward, C. H., Chandrasekaran, S., Stubbs, L. J., & Robinson, G. E. (2018, Jan). Honey bee neurogenomic responses to affiliative and agonistic social interactions. *Genes Brain Behav, 18*(1), e12509. https://doi.org/10.1111/gbb.12509

Shuel, R., & Dixon, S. (1959). Studies in the mode of action of royal jelly in honeybee development: II. Respiration of newly emerged larvae on various substrates. *Canadian Journal of Zoology, 37*(5), 803-813.

Siddiqui, R., & Khan, N. A. (2020). Locusts: a model to investigate human disease and sickness behavior. *ACS pharmacology & translational science, 3*(6), 1423-1424.

Siefert, P., Buling, N., & Grünewald, B. (2021). Honey bee behaviours within the hive: Insights from long-term video analysis. *PLOS ONE, 16*(3), e0247323. https://doi.org/10.1371/journal.pone.0247323

Signorell, A. (2023). *DescTools: Tools for Descriptive Statistics.* In (Version 0.99.48.) R package. https://CRAN.R-project.org/package=DescTools

Sih, A., Mathot, K. J., Moirón, M., Montiglio, P.-O., Wolf, M., & Dingemanse, N. J. (2015, 2015/01/01/). Animal personality and state–behaviour feedbacks: a review and guide for empiricists. *Trends in Ecology & Evolution, 30*(1), 50-60. https://doi.org/https://doi.org/10.1016/j.tree.2014.11.004

Silk, M. J., Cant, M. A., Cafazzo, S., Natoli, E., & McDonald, R. A. (2019). Elevated aggression is associated with uncertainty in a network of dog dominance interactions. *Proceedings of the Royal Society B, 286*(1906), 20190536.

Simola, D. F., Graham, R. J., Brady, C. M., Enzmann, B. L., Desplan, C., Ray, A., Zwiebel, L. J., Bonasio, R., Reinberg, D., Liebig, J., & Berger, S. L. (2016). Epigenetic (re)programming of caste-specific behavior in the ant Camponotus floridanus. *Science, 351*(6268), aac6633. https://doi.org/10.1126/science.aac6633

Simone-Finstrom, M. (2017, 2017/01/02). Social Immunity and the Superorganism: Behavioral Defenses Protecting Honey Bee Colonies from Pathogens and Parasites. *Bee World, 94*(1), 21-29. https://doi.org/10.1080/0005772X.2017.1307800

Simons, M., & Tibbetts, E. A. (2019). Insects as models for studying the evolution of animal cognition. *Current Opinion in Insect Science, 34*, 117-122.

Simpson, S. J., Despland, E., Hägele, B. F., & Dodgson, T. (2001). Gregarious behavior in desert locusts is evoked by touching their back legs. *Proceedings of the National Academy of Sciences, 98*(7), 3895-3897. https://doi.org/10.1073/pnas.071527998

Sinha, S., Jones, B. M., Traniello, I. M., Bukhari, S. A., Halfon, M. S., Hofmann, H. A., Huang, S., Katz, P. S., Keagy, J., Lynch, V. J., Sokolowski, M. B., Stubbs, L. J., Tabe-Bordbar, S., Wolfner, M. F., & Robinson, G. E. (2020, Sep 22). Behavior-related gene regulatory networks: A new level of organization in the brain. *Proc Natl Acad Sci U S A, 117*(38), 23270-23279. https://doi.org/10.1073/pnas.1921625117

Slessor, K. N., Winston, M. L., & Le Conte, Y. (2005, 2005/11/01). Pheromone Communication in the Honeybee (Apis mellifera L.). *Journal of Chemical Ecology, 31*(11), 2731-2745. https://doi.org/10.1007/s10886-005-7623-9

Smallwood, S. A., & Kelsey, G. (2012, Jan). De novo DNA methylation: a germ cell perspective. *Trends Genet, 28*(1), 33-42. https://doi.org/10.1016/j.tig.2011.09.004

Smith, C. R., Anderson, K. E., Tillberg, C. V., Gadau, J., & Suarez, A. V. (2008). Caste Determination in a Polymorphic Social Insect: Nutritional, Social, and Genetic Factors. *The American Naturalist, 172*(4), 497-507. https://doi.org/10.1086/590961

Smith, K. M., Loh, E. H., Rostal, M. K., Zambrana-Torrelio, C. M., Mendiola, L., & Daszak, P. (2013, 2013/12/01). Pathogens, Pests, and Economics: Drivers of Honey Bee Colony Declines and Losses. *EcoHealth, 10*(4), 434-445. https://doi.org/10.1007/s10393-013-0870-2

Snell-Rood, E. C. (2013). An overview of the evolutionary causes and consequences of behavioural plasticity. *Animal Behaviour, 85*(5), 1004-1011.

Snell-Rood, E. C., & Moczek, A. P. (2012). Insulin signaling as a mechanism underlying developmental plasticity: the role of FOXO in a nutritional polyphenism. *PLOS ONE, 7*(4), e34857. https://doi.org/10.1371/journal.pone.0034857

Snir, O., Alwaseem, H., Heissel, S., Sharma, A., Valdés-Rodríguez, S., Carroll, T. S., Jiang, C. S., Razzauti, J., & Kronauer, D. J. C. (2022, 2022/12/01). The pupal moulting fluid has evolved social functions in ants. *Nature, 612*(7940), 488-494. https://doi.org/10.1038/s41586-022-05480-9

Snyder-Mackler, N., Burger, J. R., Gaydosh, L., Belsky, D. W., Noppert, G. A., Campos, F. A., Bartolomucci, A., Yang, Y. C., Aiello, A. E., O'Rand, A., Harris, K. M., Shively, C. A., Alberts, S. C., & Tung, J. (2020). Social determinants of health and survival in humans and other animals. *Science, 368*(6493), eaax9553. https://doi.org/doi:10.1126/science.aax9553

Southwick, E. E., & Moritz, R. F. A. (1987, 1987/09/01). Effects of meteorological factors on defensive behaviour of honey bees. *International Journal of Biometeorology, 31*(3), 259-265. https://doi.org/10.1007/BF02188929

Søvik, E., Berthier, P., Klare, W. P., Helliwell, P., Buckle, E. L., Plath, J. A., Barron, A. B., & Maleszka, R. (2018). Cocaine directly impairs memory extinction and alters brain DNA methylation dynamics in honey bees. *Frontiers in Physiology, 9*, 79.

Spear, L. P. (2013). Adolescent neurodevelopment. *Journal of adolescent health, 52*(2), S7-S13.

Spruijt, B. M., Hooff, J. A. v., & Gispen, W. H. (1992). Ethology and neurobiology of grooming behavior. *Physiological Reviews, 72*(3), 825-852. https://doi.org/10.1152/physrev.1992.72.3.825

Sripetchwandee, J., Chattipakorn, N., & Chattipakorn, S. C. (2018). Links between obesity-induced brain insulin resistance, brain mitochondrial dysfunction, and dementia. *Frontiers in endocrinology, 9*, 496.

Stamps, J. A., & Biro, P. A. (2016). Personality and individual differences in plasticity. *Current opinion in behavioral sciences, 12*, 18-23.

Stamps, J. A., & Frankenhuis, W. E. (2016, Apr). Bayesian Models of Development. *Trends Ecol Evol, 31*(4), 260-268. https://doi.org/10.1016/j.tree.2016.01.012

Starks, P. T., Blackie, C. A., & Seeley, T. D. (2000). Fever in honeybee colonies. *Naturwissenschaften, 87*(5), 229-231.

Stay, B., & Coop, A. C. (1974, 1974/01/01/). 'Milk' secretion for embryogenesis in a viviparous cockroach. *Tissue and Cell, 6*(4), 669-693. https://doi.org/https://doi.org/10.1016/0040-8166(74)90009-3

Stein, L. R., Bukhari, S. A., & Bell, A. M. (2018). Personal and transgenerational cues are nonadditive at the phenotypic and molecular level. *Nature Ecology and Evolution, 2*, 1306-1311.

Stengl, M. (2010). Pheromone transduction in moths. *Frontiers in cellular neuroscience, 4*, 133.

Taborsky, B., & Oliveira, R. F. (2012, Dec). Social competence: an evolutionary approach. *Trends Ecol Evol, 27*(12), 679-688. https://doi.org/10.1016/j.tree.2012.09.003

Taborsky, M., & Brockmann, H. J. (2010). Alternative reproductive tactics and life history phenotypes. In *Animal behaviour: evolution and mechanisms* (pp. 537-586). Springer.

Tabuchi, M., Sakurai, T., Mitsuno, H., Namiki, S., Minegishi, R., Shiotsuki, T., Uchino, K., Sezutsu, H., Tamura, T., Haupt, S. S., Nakatani, K., & Kanzaki, R. (2013). Pheromone responsiveness threshold depends on temporal integration by antennal lobe projection neurons. *Proceedings of the National Academy of Sciences, 110*(38), 15455-15460. https://doi.org/doi:10.1073/pnas.1313707110

Tan, K., Chen, W., Dong, S., Liu, X., Wang, Y., & Nieh, J. C. (2015, 2015/06/18). A neonicotinoid impairs olfactory learning in Asian honey bees (Apis cerana) exposed as larvae or as adults. *Scientific Reports, 5*(1), 10989. https://doi.org/10.1038/srep10989

Teicher, M. H., Samson, J. A., Anderson, C. M., & Ohashi, K. (2016, Sep 19). The effects of childhood maltreatment on brain structure, function and connectivity. *Nat Rev Neurosci, 17*(10), 652-666. https://doi.org/10.1038/nrn.2016.111

Terao, K., & Mizunami, M. (2017). Roles of dopamine neurons in mediating the prediction error in aversive learning in insects. *Scientific Reports, 7*(1), 14694.

Tessmar-Raible, K., Raible, F., Christodoulou, F., Guy, K., Rembold, M., Hausen, H., & Arendt, D. (2007, 2007/06/29/). Conserved Sensory-Neurosecretory Cell Types in Annelid and Fish Forebrain: Insights into Hypothalamus Evolution. *Cell, 129*(7), 1389-1400. https://doi.org/https://doi.org/10.1016/j.cell.2007.04.041

Theis, F. J., Ugelvig, L. V., Marr, C., & Cremer, S. (2015). Opposing effects of allogrooming on disease transmission in ant societies. *Philosophical Transactions of the Royal Society B: Biological Sciences, 370*(1669), 20140108. https://doi.org/doi:10.1098/rstb.2014.0108

Theraulaz, G., Bonabeau, E., & Denuebourg, J.-N. (1998). Response threshold reinforcements and division of labour in insect societies. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 265*(1393), 327-332. https://doi.org/doi:10.1098/rspb.1998.0299

Thompson, R. F., & Spencer, W. A. (1966). Habituation: a model phenomenon for the study of neuronal substrates of behavior. *Psychological review, 73*(1), 16.

Tissot, M., & Stocker, R. F. (2000). Metamorphosis in *Drosophila* and other insects: the fate of neurons throughout the stages. *Progress in Neurobiology, 62*(89-111).

Tizard, I. (2008). Sickness behavior, its mechanisms and significance. *Animal health research reviews, 9*(1), 87-99.

Toth, A. L., & Robinson, G. E. (2007, Jul). Evo-devo and the evolution of social behavior. *Trends Genet, 23*(7), 334-341. https://doi.org/10.1016/j.tig.2007.05.001

Toth, A. L., Tooker, J. F., Radhakrishnan, S., Minard, R., Henshaw, M. T., & Grozinger, C. M. (2014). Shared genes related to aggression, rather than chemical communication, are associated with reproductive dominance in paper wasps (*Polistes metricus*). *BMC Genomics, 15*(75), 1-14.

Traynor, K. S., Le Conte, Y., & Page, R. E. (2014, 2014/12/01). Queen and young larval pheromones impact nursing and reproductive physiology of honey bee (Apis mellifera) workers. *Behavioral Ecology and Sociobiology, 68*(12), 2059-2073. https://doi.org/10.1007/s00265-014-1811-y

Tremmel, M., & Müller, C. (2012). Insect personality depends on environmental conditions. *Behavioral Ecology, 24*(2), 386-392. https://doi.org/10.1093/beheco/ars175

Trimmer, P. C., Houston, A. I., Marshall, J. A., Mendl, M. T., Paul, E. S., & McNamara, J. M. (2011). Decision-making under uncertainty: biases and Bayesians. *Animal cognition, 14*(4), 465-476.

Tripet, F., Jacot, A., & Richner, H. (2002). LARVAL COMPETITION AFFECTS THE LIFE HISTORIES AND DISPERSAL BEHAVIOR OF AN AVIAN ECTOPARASITE. *Ecology, 83*(4), 935-945. https://doi.org/10.1890/0012-9658(2002)083[0935:Lcatlh]2.0.Co;2

Truman, J. W., & Bate, M. (1988). Spatial and temporal patterns of neurogenesis in the central nervous system of Drosophila melanogaster. *Developmental biology, 125*(1), 145-157.

Tully, T., Cambiazo, V., & Kruse, L. (1994). Memory through metamorphosis in normal and mutant Drosophila. *The Journal of Neuroscience, 14*(1), 68-74. https://doi.org/10.1523/jneurosci.14-01-00068.1994

Valtorta, N. K., Kanaan, M., Gilbody, S., Ronzi, S., & Hanratty, B. (2016). Loneliness and social isolation as risk factors for coronary heart disease and stroke: systematic review and meta-analysis of longitudinal observational studies. *Heart, 102*(13), 1009-1016.

Van Os, J., Kenis, G., & Rutten, B. P. (2010). The environment and schizophrenia. *Nature, 468*(7321), 203-212.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., & Speleman, F. (2002, 2002/06/18). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology, 3*(7), research0034.0031. https://doi.org/10.1186/gb-2002-3-7-research0034

Vaudo, A. D., Patch, H. M., Mortensen, D. A., Tooker, J. F., & Grozinger, C. M. (2016). Macronutrient ratios in pollen shape bumble bee (Bombus impatiens) foraging strategies and floral preferences. *Proceedings of the National Academy of Sciences, 113*(28), E4035-E4042. https://doi.org/doi:10.1073/pnas.1606101113

Vaudo, A. D., Tooker, J. F., Patch, H. M., Biddinger, D. J., Coccia, M., Crone, M. K., Fiely, M., Francis, J. S., Hines, H. M., Hodges, M., Jackson, S. W., Michez, D., Mu, J., Russo, L., Safari, M., Treanore, E. D., Vanderplanck, M., Yip, E., Leonard, A. S., & Grozinger, C. M. (2020). Pollen Protein: Lipid Macronutrient Ratios May Guide

Broad Patterns of Bee Species Floral Preferences. *Insects, 11*(2), 132. https://www.mdpi.com/2075-4450/11/2/132

Vea, I. M., & Minakuchi, C. (2020). Atypical insects: molecular mechanisms of unusual life history strategies. *Current Opinion in Insect Science, 43*, 46-53.

Videla, M., Valladares, G., & Salvo, A. (2010). Differential effects of experience on feeding and ovipositing preferences of a polyphagous leafminer. *Entomologia Experimentalis et Applicata, 137*(2), 184-192. https://doi.org/https://doi.org/10.1111/j.1570-7458.2010.01053.x

Vitt, H. H., & Hartfelder, K. (1998, 1998/10/01/). Scientific Note neurogenesis detected by BrdU incorporation in brains of larval honey bees, apis mellifera L. (HYMENOPTERA : APIDAE). *International Journal of Insect Morphology and Embryology, 27*(4), 351-354. https://doi.org/https://doi.org/10.1016/S0020-7322(98)00028-2

Vleugels, R., Verlinden, H., & Vanden Broeck, J. (2015). Serotonin, serotonin receptors and their actions in insects. *Neurotransmitter, 2*, 1-14.

von Frisch, K. (1954). *The Dancing Bees*.

Vu, V. Q. (2011). *ggbiplot: A ggplot2 based biplot.* In http://github.com/vqv/ggbiplot

Walters, E., Illich, P., Weeks, J., & Lewin, M. (2001). Defensive responses of larval Manduca sexta and their sensitization by noxious stimuli in the laboratory and field. *Journal of Experimental Biology, 204*(3), 457-469. https://doi.org/10.1242/jeb.204.3.457

Walzer, A., & Schausberger, P. (2011, 2011/01/01/). Threat-sensitive anti-intraguild predation behaviour: maternal strategies to reduce offspring predation risk in mites. *Animal Behaviour, 81*(1), 177-184. https://doi.org/https://doi.org/10.1016/j.anbehav.2010.09.031

Wang, H., Liu, Z., Wang, Y., Ma, L., Zhang, W., & Xu, B. (2020). Genome-Wide Differential DNA Methylation in Reproductive, Morphological, and Visual System Differences Between Queen Bee and Worker Bee (Apis mellifera). *Frontiers in Genetics, 11*, 770.

Wang, Y., Campbell, J. B., Kaftanoglu, O., Page, R. E., Jr, Amdam, G. V., & Harrison, J. F. (2016). Larval starvation improves metabolic response to adult starvation in honey

bees (Apis mellifera L.). *Journal of Experimental Biology, 219*(7), 960-968. https://doi.org/10.1242/jeb.136374

Wang, Y., Kaftanoglu, O., Brent, C. S., Page, R. E., & Amdam, G. V. (2016). Starvation stress during larval development facilitates an adaptive response in adult worker honey bees (Apis mellifera L.). *The Journal of Experimental Biology, 219*(7), 949-959. https://doi.org/10.1242/jeb.130435

Wang, Y., Kaftanoglu, O., Fondrk, M. K., & Page, R. E. (2014, 2014/06/01/). Nurse bee behaviour manipulates worker honeybee (Apis mellifera L.) reproductive development. *Animal Behaviour, 92*, 253-261. https://doi.org/https://doi.org/10.1016/j.anbehav.2014.02.012

Wang, Y., Ma, L., Zhang, W., Cui, X., Wang, H., & Xu, B. (2016, 2016/01/01). Comparison of the nutrient composition of royal jelly and worker jelly of honey bees (Apis mellifera). *Apidologie, 47*(1), 48-56. https://doi.org/10.1007/s13592-015-0374-x

Watanabe, M. E. (1994). Pollination Worries Rise As Honey Bees Decline. *Science, 265*(5176), 1170-1170. https://doi.org/doi:10.1126/science.265.5176.1170

Waterson, R. H., Lander, E. S., Wilson, R. K., The Chimpanzee, S., & Analysis, C. (2005, 2005/09/01). Initial sequence of the chimpanzee genome and comparison with the human genome. *Nature, 437*(7055), 69-87. https://doi.org/10.1038/nature04072

Watson, A. H. D. (1992, 1992/10/01/). Presynaptic modulation of sensory afferents in the invertebrate and vertebrate nervous system. *Comparative Biochemistry and Physiology Part A: Physiology, 103*(2), 227-239. https://doi.org/https://doi.org/10.1016/0300-9629(92)90573-9

Watson, R. E., DeSesso, J. M., Hurtt, M. E., & Cappon, G. D. (2006). Postnatal growth and morphological development of the brain: a species comparison. *Birth Defects Research Part B: Developmental and Reproductive Toxicology, 77*(5), 471-484.

Weary, D. M., Huzzey, J. M., & von Keyserlingk, M. A. G. (2009). BOARD-INVITED REVIEW: Using behavior to predict and identify ill health in animals1. *Journal of Animal Science, 87*(2), 770-777. https://doi.org/10.2527/jas.2008-1297

Weeks, J., & Truman, J. (1986). Hormonally mediated reprogramming of muscles and motoneurones during the larval-pupal transformation of the tobacco hornworm, Manduca sexta. *Journal of Experimental Biology, 125*(1), 1-13.

Wegerhoff, R. (1999). GABA and serotonin immunoreactivity during postembryonic brain development in the beetle Tenebrio molitor. *Microscopy Research and Technique, 45*(3), 154-164. https://doi.org/10.1002/(sici)1097-0029(19990501)45:3<154::Aid-jemt3>3.0.Co;2-5

Weiner, S. A., & Toth, A. L. (2012). Epigenetics in social insects: a new direction for understanding the evolution of castes. *Genetics research international, 2012*.

Weinstock, G. M., Robinson, G. E., Gibbs, R. A., Weinstock, G. M., Weinstock, G. M., Robinson, G. E., Worley, K. C., Evans, J. D., Maleszka, R., Robertson, H. M., Weaver, D. B., Beye, M., Bork, P., Elsik, C. G., Evans, J. D., Hartfelder, K., Hunt, G. J., Robertson, H. M., Robinson, G. E., Maleszka, R., Weinstock, G. M., Worley, K. C., Zdobnov, E. M., Hartfelder, K., Amdam, G. V., Bitondi, M. M. G., Collins, A. M., Cristino, A. S., Evans, J. D., Michael, H., Lattorff, G., Lobo, C. H., Moritz, R. F. A., Nunes, F. M. F., Page, R. E., Simões, Z. L. P., Wheeler, D., Carninci, P., Fukuda, S., Hayashizaki, Y., Kai, C., Kawai, J., Sakazume, N., Sasaki, D., Tagami, M., Maleszka, R., Amdam, G. V., Albert, S., Baggerman, G., Beggs, K. T., Bloch, G., Cazzamali, G., Cohen, M., Drapeau, M. D., Eisenhardt, D., Emore, C., Ewing, M. A., Fahrbach, S. E., Forêt, S., Grimmelikhuijzen, C. J. P., Hauser, F., Hummon, A. B., Hunt, G. J., Huybrechts, J., Jones, A. K., Kadowaki, T., Kaplan, N., Kucharski, R., Leboulle, G., Linial, M., Littleton, J. T., Mercer, A. R., Page, R. E., Robertson, H. M., Robinson, G. E., Richmond, T. A., RodriguezZas, S. L., Rubin, E. B., Sattelle, D. B., Schlipalius, D., Schoofs, L., Shemesh, Y., Sweedler, J. V., Velarde, R., Verleyen, P., Vierstraete, E., Williamson, M. R., Beye, M., Ament, S. A., Brown, S. J., Corona, M., Dearden, P. K., Dunn, W. A., Elekonich, M. M., Elsik, C. G., Forêt, S., Fujiyuki, T., Gattermeier, I., Gempe, T., Hasselmann, M., Kadowaki, T., Kage, E., Kamikouchi, A., Kubo, T., Kucharski, R., Kunieda, T., Lorenzen, M., Maleszka, R., Milshina, N. V., Morioka, M., Ohashi, K., Overbeek, R., Page, R. E., Robertson, H. M., Robinson, G. E., Ross, C. A., Schioett, M., Shippy, T., Takeuchi, H., Toth, A. L., Willis, J. H., Wilson, M. J., Robertson, H. M., Zdobnov, E. M., Bork, P., Elsik, C. G., Gordon, K. H. J., Letunic, I., Hackett, K., Peterson, J., Felsenfeld, A., Guyer, M., Solignac, M., Agarwala, R., Cornuet, J. M., Elsik, C. G., Emore, C., Hunt, G. J., Monnerot, M., Mougel, F., Reese, J. T., Schlipalius, D., Vautrin, D., Weaver, D. B., Gillespie, J. J., Cannone, J. J., Gutell, R. R., Johnston, J. S., Elsik, C. G., Cazzamali, G., Eisen, M. B., Grimmelikhuijzen, C. J. P., Hauser, F., Hummon, A. B., Iyer, V. N., Iyer, V., Kosarev, P., Mackey, A. J., Maleszka, R., Reese, J. T., Richmond, T. A., Robertson, H. M., Solovyev, V., Souvorov, A., Sweedler, J. V., Weinstock, G. M., Williamson, M. R., Zdobnov, E. M., Evans, J. D., Aronstein, K. A., Bilikova, K., Chen, Y. P., Clark, A. G., Decanini, L. I., Gelbart, W. M., Hetru, C., Hultmark, D., Imler, J.-L., Jiang, H., Kanost, M., Kimura, K., Lazzaro, B. P., Lopez, D. L., Simuth, J., Thompson, G. J., Zou, Z., De Jong, P., Sodergren, E., Csűrös, M., Milosavljevic, A., Johnston, J. S., Osoegawa, K., Richards, S., Shu, C.-L., Weinstock, G. M., Elsik, C. G., Duret, L., Elhaik, E., Graur, D., Reese, J. T., Robertson, H. M., Robertson, H. M., Elsik, C. G., Maleszka, R., Weaver, D. B., Amdam, G. V., Anzola, J. M., Campbell, K. S., Childs, K. L.,

Collinge, D., Crosby, M. A., Dickens, C. M., Elsik, C. G., Gordon, K. H. J., Gramates, L. S., Grozinger, C. M., Jones, P. L., Jorda, M., Ling, X., Matthews, B. B., Miller, J., Milshina, N. V., Mizzen, C., Peinado, M. A., Reese, J. T., Reid, J. G., Robertson, H. M., Robinson, G. E., Russo, S. M., Schroeder, A. J., St Pierre, S. E., Wang, Y., Zhou, P., Robertson, H. M., Agarwala, R., Elsik, C. G., Milshina, N. V., Reese, J. T., Weaver, D. B., Worley, K. C., Childs, K. L., Dickens, C. M., Elsik, C. G., Gelbart, W. M., Jiang, H., Kitts, P., Milshina, N. V., Reese, J. T., Ruef, B., Russo, S. M., Venkatraman, A., Weinstock, G. M., Zhang, L., Zhou, P., Johnston, J. S., Aquino-Perez, G., Cornuet, J. M., Monnerot, M., Solignac, M., Vautrin, D., Whitfield, C. W., Behura, S. K., Berlocher, S. H., Clark, A. G., Gibbs, R. A., Johnston, J. S., Sheppard, W. S., Smith, D. R., Suarez, A. V., Tsutsui, N. D., Weaver, D. B., Wei, X., Wheeler, D., Weinstock, G. M., Worley, K. C., Havlak, P., The Honeybee Genome Sequencing, C., Overall project, l., Principal, i., Community, c., Annotation section, l., Caste, d., reproduction, sequencing, E. S. T., Brain, behaviour, Development, metabolism, Comparative, evolutionary, a., Funding agency, m., Physical, genetic, m., Ribosomal, R. N. A. g., related retrotransposable, e., Gene, p., consensus gene, s., Honeybee, d., immunity, construction, B. A. f. l., analysis, content, G. C., Transposable, e., Gene regulation including mi, R. N. A., Rnai, Superscaffold, a., Data, m., Chromosome, s., Population, g., Snps, & Genome, a. (2006, 2006/10/01). Insights into social insects from the genome of the honeybee Apis mellifera. *Nature, 443*(7114), 931-949. https://doi.org/10.1038/nature05260

Wessnitzer, J., & Webb, B. (2006). Multimodal sensory integration in insects—towards insect brain control architectures. *Bioinspiration & biomimetics, 1*(3), 63.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.

West-Eberhard, M. J. (2005). Developmental plasticity and the origin of species differences. *Proceedings of the National Academy of Sciences, 102*(suppl 1), 6543-6549.

Wheeler, D. E., & Nijhout, H. F. (1984, 1984/01/01/). Soldier determination in Pheidole bicarinata: Inhibition by adult soldiers. *Journal of Insect Physiology, 30*(2), 127-135. https://doi.org/https://doi.org/10.1016/0022-1910(84)90116-1

Whickam, H. (2016). *ggplot2: Elegant Graphics for Data Analysis.* In

Wiley, R. H. (2006). Signal Detection and Animal Communication. In *Advances in the Study of Behavior* (Vol. 36, pp. 217-247). Academic Press. https://doi.org/https://doi.org/10.1016/S0065-3454(06)36005-6

Wilke, C. O. (2020). *cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'.* In https://CRAN.R-project.org/package=cowplot

Willingham, R., Klopchin, J., & Ellis, J. (2000). Robbing behavior in honey bees. *Behav. Ecol., 11*(3), 326-333.

Wilson, E. O. (1985). The Sociogenesis of Insect Colonies. *Science, 228*(4707), 1489-1495. https://doi.org/doi:10.1126/science.228.4707.1489

Wilson, S. N., Sindi, S. S., Brooks, H. Z., Hohn, M. E., Price, C. R., Radunskaya, A. E., Williams, N. D., & Fefferman, N. H. (2020). How emergent social patterns in allogrooming combat parasitic infections. *Frontiers in Ecology and Evolution, 8*, 54.

Winfree, R., Gross, B. J., & Kremen, C. (2011, 2011/11/15/). Valuing pollination services to agriculture. *Ecological Economics, 71*, 80-88. https://doi.org/https://doi.org/10.1016/j.ecolecon.2011.08.001

Winston, M. L. (1992). The biology and management of Africanized honey bees. *Annual Review of Entomology, 37*(1), 173-193.

Wisenden, B. D., Lanfranconi-Izawa, T. L., & Keenleyside, M. H. A. (1995, 1995/03/01/). Fin digging and leaf lifting by the convict cichlid, Cichlasoma nigrofasciatum: examples of parental food provisioning. *Animal Behaviour, 49*(3), 623-631. https://doi.org/https://doi.org/10.1016/0003-3472(95)80195-2

Wittwer, B., Hefetz, A., Simon, T., Murphy, L. E., Elgar, M. A., Pierce, N. E., & Kocher, S. D. (2017). Solitary bees reduce investment in communication compared with their social relatives. *Proceedings of the National Academy of Sciences, 114*(25), 6569-6574.

Wolbert, P., & Kubbies, M. (1983). The cell cycle of epidermal cells during reprogramming in the pupa of Galleria. *Journal of Insect Physiology, 29*(10), 787-794.

Wolf, C., & Linden, D. E. J. (2011). Biological pathways to adaptability – interactions between genome, epigenome, nervous system and environment for adaptive behavior. *Genes, Brain and Behavior, 11*(1), 3-28. https://doi.org/10.1111/j.1601-183X.2011.00752.x

Wray, M. K., Mattila, H. R., & Seeley, T. D. (2011, 2011/03/01/). Collective personalities in honeybee colonies are linked to colony fitness. *Animal Behaviour, 81*(3), 559-568. https://doi.org/https://doi.org/10.1016/j.anbehav.2010.11.027

Wu, F., Ma, C., Han, B., Meng, L., Hu, H., Fang, Y., Feng, M., Zhang, X., Rueppell, O., & Li, J. (2019). Behavioural, physiological and molecular changes in alloparental caregivers may be responsible for selection response for female reproductive investment in honey bees. *Molecular Ecology, 28*(18), 4212-4227.

Wu, Q., & Brown, M. R. (2006). SIGNALING AND FUNCTION OF INSULIN-LIKE PEPTIDES IN INSECTS. *Annual Review of Entomology, 51*(1), 1-24. https://doi.org/10.1146/annurev.ento.51.110104.151011

Yagound, B., Remnant, E. J., Buchmann, G., & Oldroyd, B. P. (2020, Nov 30). Intergenerational transfer of DNA methylation marks in the honey bee. *Proc Natl Acad Sci U S A*. https://doi.org/10.1073/pnas.2017094117

Yan, H., Simola, D. F., Bonasio, R., Liebig, J., Berger, S. L., & Reinberg, D. (2014, 2014/10/01). Eusocial insects as emerging models for behavioural epigenetics. *Nature Reviews Genetics, 15*(10), 677-688. https://doi.org/10.1038/nrg3787

Yang, E.-C., Chang, H.-C., Wu, W.-Y., & Chen, Y.-W. (2012). Impaired Olfactory Associative Behavior of Honeybee Workers Due to Contamination of Imidacloprid in the Larval Stage. *PLOS ONE, 7*(11), e49472. https://doi.org/10.1371/journal.pone.0049472

Yang, X., & Cox-Foster, D. (2007). Effects of parasitization by Varroa destructor on survivorship and physiological traits of Apis mellifera in correlation with viral incidence and microbial challenge. *Parasitology, 134*(3), 405-412.

Yartsev, M. M. (2017). The emperor's new wardrobe: rebalancing diversity of animal models in neuroscience research. *Science, 358*(6362), 466-469.

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC bioinformatics, 13*, 1-11.

Zarchin, S., Dag, A., Salomon, M., Hendriksma, H. P., & Shafir, S. (2017, 2017/11/09). Honey bees dance faster for pollen that complements colony essential fatty acid deficiency. *Behavioral Ecology and Sociobiology, 71*(12), 172. https://doi.org/10.1007/s00265-017-2394-1

Zemach, A., McDaniel, I. E., Silva, P., & Zilberman, D. (2010). Genome-Wide Evolutionary Analysis of Eukaryotic DNA Methylation. *Science, 328*(5980), 916-919. https://doi.org/10.1126/science.1186366

Zera, A. J., & Denno, R. F. (1997, 1997/01/01). PHYSIOLOGY AND ECOLOGY OF DISPERSAL POLYMORPHISM IN INSECTS. *Annual Review of Entomology, 42*(1), 207-230. https://doi.org/10.1146/annurev.ento.42.1.207

Zhang, C.-X., Brisson, J. A., & Xu, H.-J. (2019). Molecular mechanisms of wing polymorphism in insects. *Annual Review of Entomology, 64*, 297-314.

Zhao, C., Deng, W., & Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell, 132*(4), 645-660.

Zill, S. N. (1986). A model of pattern generation of cockroach walking reconsidered. *Journal of Neurobiology, 17*(4), 317-328. https://doi.org/10.1002/neu.480170406

# VITA

1. Educational institutions attended:
    - The University of Texas at Austin (2013-2016)
        - Bachelor of Science, Biology (*graduated with Honors*)
        - Bachelor of Arts, Iberian and Latin American Languages and Cultures (*graduated with High Honors*)
2. Professional positions held:
    - Field Technician, Old Dominion University (2016-2017)
    - Biological Science Aide, United States Department of Agriculture (2017)
3. Scholastic and professional honors:
    - Grants and Fellowships:
        - Animal Behavior Society Student Research Grant (2019)
        - Richards Graduate Student Research Activity Award (2019)
        - *Honorable Mention*, NSF Graduate Research Fellowship (2020)
        - Grains in Aid of Research (2020)
        - University of Kentucky Presidential Fellowship (2021)
    - Marlene Zuk Best Student Presentation Award—Society of Integrative and Comparative Biology Annual Meeting
4. Publications:

**Westwick, R. R.,** Brackett, G. P., Brown, C. E., Ison, B. J., & Rittschof, C. C. Response to competing conspecific cues depends on social context in the honey bee (*Apis mellifera*). *In press*.

*Napier, T. C, **Westwick, R. R.**, Kane, C. W., & Rittschof, C. C. (2023). Evaluating the cues that coordinate a shift towards the robbing foraging tactic in the honey bee (*Apis mellifera*). *Behav Ecol Sociobiol* **77**, 46.

*Foose, A. M., **Westwick, R. R.**, Vengarai, M., & Rittschof, C. C. (2022). The survival consequences of grooming in the honey bee Apis mellifera. *Insectes Sociaux*, *69*(2), 279-287.

**Westwick, R. R.**, & Rittschof, C. C. (2021). Insects provide unique systems to investigate how early-life experience alters the brain and behavior. *Frontiers in Behavioral Neuroscience*, *15*, 61.

Burkhard, T. T., **Westwick, R. R.**, & Phelps, S. M. (2018). Adiposity signals predict vocal effort in Alston's singing mice. *Proceedings of the Royal Society B: Biological Sciences, 285*(1877).

*First author is an undergraduate mentee of R. Westwick

5. Rebecca R. Westwick