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Protein Nutrition and Immunity in Male Bumblebees (*Bombus impatiens*)

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PROTEIN NUTRITION AND IMMUNITY IN MALE BUMBLEBEES (*BOMBUS IMPATIENS*)

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Abstract

Male bumble bees play a vital but understudied role in their colonies. They are essential to colony reproduction and success, despite not emerging until the end of the colony's life cycle. Even so, the biology of male bumble bees is not well understood, which leaves our understanding of colony health incomplete. This knowledge gap is important because bumble bee populations are declining, which threatens the ecosystems and industries which rely on them for pollination. This study aimed to understand how two understudied factors of bumble bee health—diet and immunity—are related in males. Pollen, a bee's main source of protein, has been shown to be an important contributor to the overall health of bees. However, the effect of protein diet on various immune responses is varied. *Bombus impatiens* males produced by microcolonies were placed into two treatment groups, one with access to protein and one without access to protein. A nylon filament immune challenge was used to assess the encapsulation and melanization immune response in both groups. RT-qPCR was used to compare expression of immune genes involved in melanization and antimicrobial peptides (AMPs). A mixed effects model revealed the strength of encapsulation and melanization was statistically similar between the two groups. These findings suggest the response of cellular immune function to protein deprivation in male bumble bees may be similar to what has been previously observed in females. Further scientific understanding of the health of males could lead to overall heightened colony success, preserving bumble bees and the pollination services they provide for both the environment and agriculture.

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Introduction

Our knowledge of how male bumble bee biology differs from that of female bumble bees is limited. Female workers take care of the majority of the colony's needs and perform most of the direct pollination services. However, males are also essential to the reproductive success and life cycle of a colony. They mate with queens from other colonies, contributing to the fitness of the colony, perpetuating the colony's genetic legacy and supporting overall population growth. In addition, males of some species have been shown to perform tasks in the nest such as incubation of brood (Cameron, 1985). Via these roles in the colony lifecycle, males contribute to pollination services indirectly. Understanding the factors that affect male bumble bee health is thus an important component in protecting these important pollinators, many of which are experiencing population decline (Belsky et al., 2020, Cameron and Sadd, 2020). Specifically, research on the nutritional requirements and immunocompetence of male bumble bees is greatly needed.

Nutrition is a significant factor that influences bee health including body size, immunity, reproduction, and the development of sexual organs (Schmid-Hempel and Schmid-Hempel, 1998, Ponton et al., 2011, Pain, 1968, Pech-May et al., 2012). Male honey bees consume pollen, which is the primary source of protein for bees, for about the first 6 days of their adult life. Pollen consumption peaks after 3 or 4 days, after which point they rely almost solely on carbohydrates for energy to fly (Free, 1957, Szolderits and Crailsheim 1993). This early-life protein consumption may be necessary for reproductive maturity and survival. Supplemental protein in honey bees is associated with increased sperm viability and semen volume when given to the colony before the production of males (Rousseau & Giovenazzo, 2016). Honey bee workers survive longer at lower protein-carbohydrate ratios, suggesting overall more carbohydrates are needed in their diet than protein (Pirk et al., 2010). Similarly, *B. terrestris*

workers preferred protein carbohydrate ratios of roughly 1:150 (Stabler et al., 2015). *Bombus impatiens* foragers preferred plants with higher protein-lipid ratios and tended to choose pollen with 5:1 or 10:1 ratios (Vaudo et al., 2016a). Both *B. impatiens* and *B. terrestris* workers alter their consumption of certain macronutrients in order to reach protein lipid ratios of 12:1 and 14:1 respectively (Vaudo et al., 2016b). These studies suggest that bee protein consumption is important to bee health, and tightly regulated according to functional requirements. However, more research on the specific nutritional requirements of male bumble bees is needed (Belsky et al., 2020).

Immunity is another vital factor for bumble bee health and reproductive abilities. Being unable to fight off pathogens leads to disease, death, and ultimately lower reproductive outcomes. Pathogens are one of the major contributing factors to declines in bee populations (Zhao and Liu, 2022). Immune responses in bees are generally initiated through receptor-mediated recognition of pathogen-associated molecular patterns (PAMP) on pathogens or foreign bodies (Rosales, 2017). Lipopolysaccharide (LPS), a component of the outer membrane in Gram-negative bacteria, is one such example of a PAMP. LPS is able to prompt an immune reaction even in the absence of an actual pathogen (Moret and Schmid-Hempel, 2000). Recognition of a foreign pattern can trigger a variety of innate immune effector mechanisms. Cellular immune responses such as phagocytosis, nodulation, encapsulation, and melanization require the action of hemocytes. The humoral immune response generally involves formation of antimicrobial peptides (AMP) such as defensin, abaecin, and hymenoptaecin which are formed through a variety of signaling pathways (Rosales, 2017). The main viral defense in insects is RNA interference (Degrandi-Hoffman and Chen, 2015). Interestingly, bumble bees, like other eusocial bees, have fewer canonical immune genes than do nonsocial insects (Barribeau et al.,

2015). This fact would suggest bumble bees are more susceptible to disease, but it is thought they make up for this reduced immune array through social behaviors such as grooming which help prevent the spread of disease. (Barribeau et al., 2015).

Melanization and encapsulation are two basic, innate immune functions found in many insects, including bumble bees. Melanization is an immune response in which pathogens are coated by a layer of dark melanin. Once a foreign molecule is recognized, a serine protease cascade cleaves the enzyme prophenoloxidase (PPO). The active form of this enzyme, phenoloxidase (PO), then initiates a cascade resulting in the synthesis of the protein melanin. Melanin can cross-link with other hemolymph proteins and aggregate on the surface of the foreign body or pathogen, helping to neutralize it (Söderhäll and Cerenius, 1998, Hillyer, 2016). The melanin cascade also produces many reactive oxygen by-products, which aid in the destruction of the foreign body (Hillyer, 2016). Encapsulation is a similar and related immune response where layers of hemocytes surround and encapsulate pathogens, typically parasites too large to be phagocytosed (Rosales, 2017). These responses isolate the pathogen, which blocks it from doing further harm, and kills it via reactive oxygen species and or starvation (Hillyer, 2016). Melanization is also heavily involved in encapsulation, and the hemocytes responsible for the encapsulation process produce many of the enzymes in the melanin synthesis pathway (Hillyer, 2016). Melanization has also been shown to be important in wound healing and in the cellular immune response of nodulation in insects (Rosales, 2017).

Little research exists on male immunity, but some differences have been observed between males and females. Multiple groups have reported that the encapsulation response is higher in bumble bee workers than in males. Gerloff et al. (2003) observed this difference between sexes in *B. terrestris* using a nylon filament challenge and also observed the response

was lower in males produced later in the colony lifespan. Baer and Schmid-Hempel (2006) reported a similar difference between sexes in *B. terrestris* and observed that encapsulation was independent of body size and ploidy in males. Barribeau et al. (2015) found that expression of many immune genes was more highly induced in queens than in males when injected with *A. globiformis* and when injected with *E. coli*, and that queens experienced higher overall expression of these genes. These observations are in line with Bateman's principle of investment wherein females invest more resources into survival and longevity than males do (Rolff, 2002).

Diet and immunity likely also have synergistic effects on male bumble bee health. Immunity is an energetically costly process, and it follows that greater access to energy in the form of food will allow for stronger immune responses. Under starvation conditions, bumble bee workers experience reduced survival upon immune challenge (Moret and Schmid-Hempel, 2000). Bumble bee workers consume more honey when mounting an immune response against injected LPS (Tyler et al., 2006). Additionally, dietary protein seems to be important for the expression of many immune genes such as AMPs. Protein deprivation has been associated with lower immune gene expression in bumble bee workers (Brunner et al., 2014). Supplemental sunflower pollen upregulates expression of many immune genes upon infection, including AMPs, in workers (Giacomini et al., 2023). Conversely, protein consumption does not seem to affect the encapsulation response for worker bees. One study found no significant differences in PO activity in honey bee workers fed with pollen and those fed with no pollen (Alaux et al., 2010). Similarly, in bumble bees protein quality (type of pollen) did not have an effect on the strength of PO activity in workers (Rogers et al. 2017). However, it remains to be investigated if these trends hold in male bumble bees.

This study aimed to answer the question how does diet, specifically a lack of protein, affect the immunity of male bumble bees? If protein nutrition is important for the melanization response in male bumble bees, then male bumble bees deprived of dietary protein will experience decreased immune function compared to bees fed a normal diet. We tested this hypothesis in immune challenged males of *B. impatiens* using two methods. First, the melanization response of protein-deprived bees was measured after an immune challenge in which nylon filaments coated with LPS were inserted into the abdomen of the bees. Second, gene expression of an antimicrobial peptide and a melanin synthesis enzyme was measured using RT-qPCR. If protein deficiency is associated with decreased immunocompetence, then we predict male bees fed with low protein diets will demonstrate a decreased melanization response to the nylon filament challenge and an immune gene expression profile consistent with a decrease in immunocompetence, relative to the male bees fed a regular protein diet. This work has the potential to enhance the health and success of bumble bee colonies, reversing the alarming population declines currently being observed and leading to both healthier ecosystems and more productive agriculture.

Methods

Bee Rearing and Diet Treatments

We obtained two colonies of *B. impatiens* from Koppert Biological Systems (Howell, MI) near the beginning of their lifecycle. Colonies were kept in a climate-controlled room at 26-28 °C and 60-65% relative humidity. Colonies were also kept in the dark and red light was used when tending them. We fed the colonies sugar water (1:1 volume of sugar to distilled water) and pollen dough *ad libitum*. Honey bee pollen (Betterbee, Greenwich, NY) was ground into a fine

powder using a coffee grinder and mixed with sugar water in a 7.5:1 pollen to sugar water ratio to produce the pollen dough. Once the colony was several days old, we set up microcolonies containing three worker bees each, and provided pollen and sugar water *ad libitum*. Pollen was formed into circular disks about the size of a quarter and placed on a white piece of cardstock attached to the bottom of one side of the cage. The pollen on the paper acts as a substrate for bees to create honeypots and lay eggs upon. We checked the microcolonies every day to monitor the egg-laying process and check for newly emerged males (Colony B: 35 males emerged, Colony C: 10 males emerged). When a male emerged, they were collected and placed into individual cages with honey bee pollen mixed into their sugar water (83.3 mg/mL). After two days, we randomly assigned males to a treatment group. Roughly equal amounts of males from each microcolony were placed in each treatment to minimize bias from genetically related individuals. The control group was given this same sugar water-protein mixture, and the protein-deprived group was given sugar water without protein in it. Bees were kept in treatment for one day.

Nylon Filament Immune Challenge

At the end of the treatment period, we exposed all individuals (n=67) to a nylon filament immune challenge. First, the bees were moved from their individual cages into 15 mL tubes and chilled on ice for 10-20 minutes to anesthetize them. Once asleep, individual bees were immobilized by cross-pinning to a silicone-filled petri dish. We sterilized the abdomen of each bee with ethanol and gently inserted a 0.8 mm sterile needle between the 3rd and 4th sternite. A nylon filament (3 mm x 0.2 mm) was dipped in 0.5 m/mL LPS (Sigma-Aldrich, Saint Louis, MO) and inserted in the same location as the needle is removed. This method exposes the

filament to the hemolymph, where melanization and encapsulation occur. A portion of the filament was left outside the bee for easy removal. We put the bees back in a 15 mL tube along with cotton dipped in sugar water. They were then placed in a 26 °C incubator for 4 hours. After the incubation, bees still alive (capable of voluntary movement, n=53) were placed in the freezer for about 5 minutes. We re-pinned them and removed the filament. The bee was immediately frozen and stored in liquid nitrogen. For long term storage, the bees were kept in a -80 °C freezer.

We then placed the removed filament next to a blank filament on a piece of photopaper on top of a grey background. Pictures were taken of 3 different sides of each filament using a Leica EZ4HD microscope (Wetzlar, Germany). We analyzed these images using the Fiji distribution of ImageJ (Schindelin et al., 2012). Analysis was done by uploading the image into the software and converting it to 16 bit grayscale. Boxes were drawn around both the blank filament and the portion of the melanized filament which was inside the bee. The grey value of all the pixels in each box were then measured to find the average grey value of each filament. The grey value of the blank filament was then subtracted from the grey value of the melanized filament to control for differences in lighting. A group of five filaments was measured at the beginning, end, and middle of the process to check for consistency in measurements. Photos from each treatment group were well-mixed during the analysis, to mitigate bias during the measurement process.

RT-qPCR

We performed all reactions according to the manufacturer's recommended protocol. We performed RNA extraction using abdomens of 16 of the frozen bees, 8 from each treatment

group. We only did 16 of the bees due to resource constraints. Extraction was done using RNeasy mini spin columns including the DNA digestion step (Qiagen, Germantown, MD). Frozen tissue was homogenized in RLT buffer using a motorized pestle. We quantified RNA extracts using a Qubit RNA HS Assay Kit (Invitrogen, Waltham, MA) and purity was assessed via nanodrop. Any samples exhibiting low 260/280 or 260/230 ratios were purified further by repeating the RNeasy Mini kit protocol. We performed reverse transcription for all samples using High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). We reverse transcribed 500 ng of RNA in a 20 mL and generated appropriate no template and negative RT controls. Primer sequences for *arginine kinase* were obtained from Amsalem et al. (2017). We designed primers for *prophenoloxidase* (*PPO*), and *abaecin* using NCBI Primer Blast and double checked their specificity using BLAST (Ye et al., 2012). We ran primer efficiency tests for the primers prior to performing RT-qPCR; all primers had 94-101% efficiency. The descriptions of these genes and primer sequences are shown in Table 1 below. We performed RT-qPCR with SYBR Green Supermix and a CFX Opus 384 machine (BioRad, Hercules, CA). 2 µL of cDNA were used in each reaction.

Statistical Analysis

For the nylon filament immune challenge, we performed data preprocessing and summary statistics in Jupyter Notebook version 6.5.2 (Kluyver et al., 2016). We did the rest of the filament analyses in R version 4.2.3 (R Core Team, 2023). Normality was assessed via an Anderson-Darling test in nortest version 1.0.4 (Gross and Ligges, 2015). Skewness and kurtosis were visualized using fitdistrplus version 1.1.8 (Delignette-Mullerand Dutang 2015). Quantile distribution was visualized using car version 3.1.0 (Fox and Weisberg, 2019). We created a

mixed effect model in lme4 version 1.1.30 (Bates et al., 2015). We added source colony and microcolony to this model as random effects. We did this because individuals originating from the same colony or microcolony are genetically related, which is important to take into account as it can create pseudoreplication. However, colony had a variance of 0, and it was removed from the final model. Plots were generated using ggplot2 in the tidyverse package version 2.0.0 (Wickham et al., 2019). We used Microsoft Excel to calculate primer efficiency and to analyze the RT-qPCR results using the delta-delta CT method (Microsoft Corporation, 2018).

Table 1

Gene	**Description**	**NCBI Accession**	**Forward Primer Sequence**	**Reverse Primer Sequence**	**Primer Efficiency**
Arginine Kinase	Reference	XM_012391881.3	GTTGGTAGGGCAGAAGGTCA	AGGTCTACCGTCGTCTGGTG	100.08% (R^2=0.992)
PPO	prophenoloxidase, melanin synthesis	XM_012387043.2	CCAGCGCATCGAAGAATAGC	GATCCTTCGTGTCAGGACGA	100.50% (R^2=0.982)
abaecin	AMP	XM_003491496	AGGAGCAAGTTGTGGAGAGAAAA	CATGACCTGGGAAGCTTGGA	94.17% (R^2=0.997)

Table containing information about the genes and primers used for RT-qPCR.

Results

A total of 67 males emerged from 16 different microcolonies over the course of three weeks. We collected these bees and placed them into treatment groups. Of these males, 53 (79.1%) survived until the end of the nylon filament challenge. There were no significant

differences in survival between the control (28/35 – 78.13% surviving) and protein deprived groups (25/32 – 82.35% surviving) (chi-sq = 0.04, p = 0.85). However, only 45 of these 53 filaments were used in the final grey value analysis, as some were lost in the removal process. Grey value is a measure of the level of greyness of a pixel. It is represented on a scale from 0 to 255 with 0 being the least grey and 255 being the most grey. The higher the grey value, the darker the filament and the stronger the melanization response of the bee. Males in the protein-deprived group had a slightly weaker melanization response than those in the control group (i.e., 10% lower grey value; Table 2), but this trend was not statistically significant (chi-sq = 1.11, p = 0.29; Fig. 1).

Eight bees in each treatment group were randomly selected for gene expression analysis and RNA extraction was successfully performed. The RT-qPCR portion of the study is still in progress.

Table 2– Mean Grey Values

Group	**Sample Size**	**Mean Grey Value**	**Standard Error**
All bees	45	43.47	2.34
Control	22	45.92	2.96
Protein-deprived	23	41.12	3.59

Table displaying summary statistics of the grey values by treatment

Figure 1- Grey Value Distribution

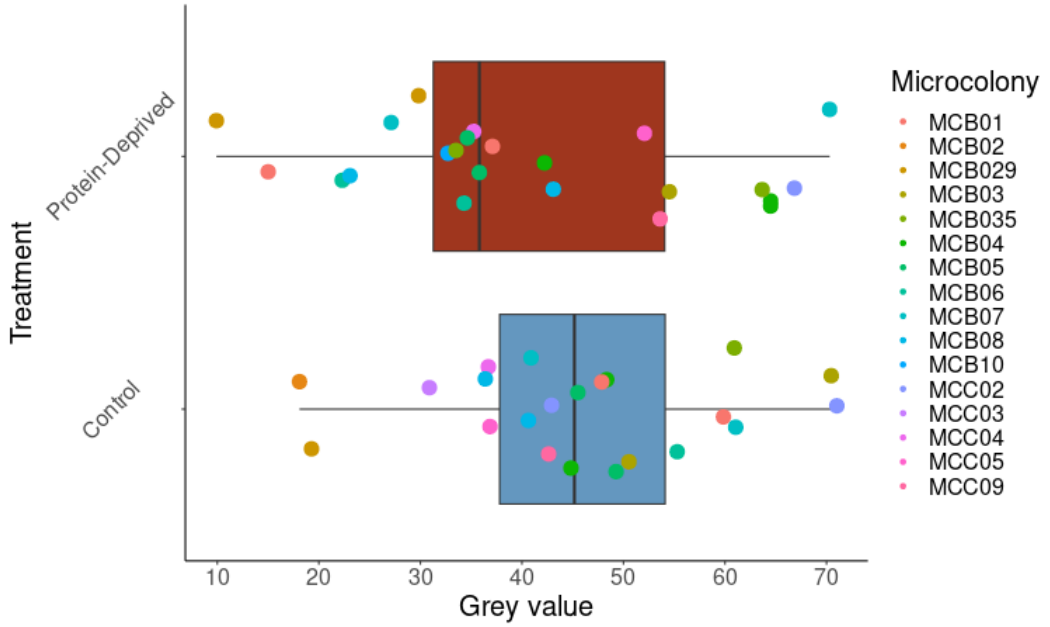

Figure 1. Grey values by treatment group. Red is the protein-deprived group and blue is the control group. Each of the data points on the plot represents a bee, colored by the microcolony they came from. The lines in the middle of each plot represent the median, or the point at which 50% of the data lies above that value and 50% lies below. The left and right bounds of each box represent the lower and upper quartiles respectively. The area of the box is the interquartile range, in which 50% of the data lies. The whiskers each represent the 25% of data at each extreme. The protein deprived group has both a lower median and greater variation compared to the control.

Discussion

In this study, we investigated the effects of protein on the immune function of male bumble bees. We used short-term protein deprivation to measure the melanization and encapsulation immune response and gene expression of multiple immune genes. We did not find evidence for a difference in the melanization and encapsulation response between the bees fed protein and the protein-deprived bees. Our results support other studies which have not found protein content or pollen quality to have a significant effect on phenoloxidase activity in workers

of both honey bees (Alaux et al., 2010) and bumble bees (Roger et al., 2017). Another study found that poor nutritional environments, where food was available only for select times, were not associated with changes in encapsulation responses as measured by filament immune challenges in *B. terrestris* workers (Schmid-Hempel and Schmid-Hempel, 1998). Furthermore, Brunner et al. (2014) reported no correlation between phenoloxidase activity and starvation, despite also finding that protein deprivation over a 24-hour period was associated with lower expression of many immune genes, including AMPs. We continued this investigation in males, and our results suggest this pattern is consistent between sexes.

Immunity is a complex system, and there are often tradeoffs between different responses (Cotter and Al Shareefi, 2022). With limited resources, an organism must allocate its resources as efficiently as it can, leading to tradeoffs which depend on the nutrients available and the immune threat present. For example, phenoloxidase activity is negatively correlated with expression of multiple antimicrobial peptides in bumble bee workers (Moret and Schmid-Hempel, 2009). Brunner et al. (2014) found similar results under protein deprivation, with AMP expression being more affected by lack of protein. There is also evidence for tradeoffs between the non-specific (encapsulation) and specific (via infection intensity) immune responses in bumble bee workers (Mallon et al., 2003). Interestingly, tradeoffs between melanization and AMPs have even been found in male bumble bees at both the phenotypic and genetic levels (Wilfert et al., 2007). It should also be noted that expression of antimicrobial peptides appears to be one of the immune responses most limited by lack of protein in the bumble bee diet (Brunner et al., 2014). On the other hand, it seems melanization and phenoloxidase activity is more dependent on carbohydrates than it is on protein (Cotter et al., 2011, Roger et al., 2017, Degrandi-Hoffman and Chen, 2015). Therefore, it is likely immune responses other than

melanization, such as AMP levels, were diminished in the bees used here. We also have not yet completed the gene expression portion of the experiment. The expression of both PPO, a melanin synthesis gene, and abaecin, an antimicrobial peptide, are being measured. Hopefully, those results will shed light on the full interaction of diet with these immune tradeoffs in male bumble bees.

There have also been differences observed in the immune response of male and female bees. Males have lower encapsulation levels than females under filament immune challenges in bumble bees (Baer and Schmid-Hempel 2006). Expression of a variety of immune genes is also lower in males than gynes in multiple bumble bee species (Barribeau et al., 2015). Another study found that supplemental sunflower pollen fed to *B. impatiens* decreased pathogen levels in females, but had no effect in males (Fowler et al., 2020). Therefore, there may be differences in the immune reaction to nutritional changes between sexes in bumble bees. According to Bateman's principle of investment, females should invest more in longevity mechanisms such as immunity, and males should invest more in short term reproductive efforts. If this principle is correct, then protein consumption in adult male bumble bees may be more important for reproductive measures, which we did not evaluate. Along these lines, one study found that protein deprivation in honey bee males after their emergence did not affect the viability of their sperm (Stürup et al., 2013). This effect may occur because spermatogenesis occurs during the larval stage, meaning that adult nutrition has no effect on the already developed sperm. However, it is still possible that dietary protein is an important limiting factor for other measures important to reproduction such as the production of semen. For example, Rousseau and Giovenazzo (2016) found that both protein and carbohydrate supplements given to a colony had a significant positive effect on the body size and semen volume of males produced from these colonies.

Further investigation into sexual differences in nutritional modulation of immunity and reproduction is warranted.

In conclusion, we did not find short term protein deprivation to have a notable effect on the encapsulation and melanization response of male bumble bees. More work is yet needed to understand the mechanisms of diet and immunity in male bumble bees, and how it differs from female bumble bees. Promising areas for continuing our investigation into male bumble bee immunocompetence include examining the effect of protein deprivation on AMPs, the effect of carbohydrate deprivation on melanization and encapsulation, and if the length of the treatment period is important. This line of research has the potential to improve bumble bee heath at the individual, colony, and population levels in the wild. It will be a step towards better management and conservation of not only these species, but the entire ecosystems they live in and the agricultural industries which rely on them.

References

Alaux, C., Ducloz, F., Crauser, D., & Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biology Letters*, *6*(4), 562–565. https://doi.org/10.1098/rsbl.2009.0986

Amsalem, E., Padilla, M., Schreiber, P. M., Altman, N. S., Hefetz, A., & Grozinger, C. M. (2017). Do Bumble Bee, Bombus impatiens, Queens Signal their Reproductive and Mating Status to their Workers? Journal of Chemical Ecology, 43(6), 563–572. https://doi.org/10.1007/s10886-017-0858-4

Baer, B., & Schmid-Hempel, P. (2006). Phenotypic variation in male and worker encapsulation response in the bumblebee Bombus terrestris. *Ecological Entomology*, *31*(6), 591–596. https://doi.org/10.1111/j.1365-2311.2006.00825.x

Barribeau, S. M., Sadd, B. M., du Plessis, L., Brown, M. J., Buechel, S. D., Cappelle, K., Carolan, J. C., Christiaens, O., Colgan, T. J., Erler, S., Evans, J., Helbing, S., Karaus, E., Lattorff, H. M. G., Marxer, M., Meeus, I., Näpflin, K., Niu, J., Schmid-Hempel, R., … Schmid-Hempel, P. (2015). A depauperate immune repertoire precedes evolution of sociality in bees. *Genome Biology*, *16*(1), 83. https://doi.org/10.1186/s13059-015-0628-y

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Belsky, J. E., Camp, A. A., & Lehmann, D. M. (2020). The Importance of Males to Bumble Bee (Bombus Species) Nest Development and Colony Viability. *Insects*, *11*(8), 506. https://doi.org/10.3390/insects11080506

Brunner, F. S., Schmid-Hempel, P., & Barribeau, S. M. (2014). Protein-poor diet reduces host-specific immune gene expression in Bombus terrestris. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1786), 20140128. https://doi.org/10.1098/rspb.2014.0128

Cameron, S. A. (1985). Brood care by male bumble bees. Proceedings of the National Academy of Sciences of the United States of America, 82(19), 6371–6373. https://doi.org/10.1073/pnas.82.19.6371

Cameron, S. A., & Sadd, B. M. (2020). Global Trends in Bumble Bee Health. *Annual Review of Entomology*, *65*(1), 209–232. https://doi.org/10.1146/annurev-ento-011118-111847

Cotter, S. C., & Al Shareefi, E. (2022). Nutritional ecology, infection and immune defence—Exploring the mechanisms. *Current Opinion in Insect Science*, *50*, 100862. https://doi.org/10.1016/j.cois.2021.12.002

Cotter, S. C., Simpson, S. J., Raubenheimer, D., & Wilson, K. (2011). Macronutrient balance mediates trade-offs between immune function and life history traits. *Functional Ecology*, *25*(1), 186–198. https://doi.org/10.1111/j.1365-2435.2010.01766.x

DeGrandi-Hoffman, G., & Chen, Y. (2015). Nutrition, immunity and viral infections in honey bees. *Current Opinion in Insect Science*, *10*, 170–176. https://doi.org/10.1016/j.cois.2015.05.007

Delignette-Muller, M. L., & Dutang, C. (2015). fitdistrplus: An R Package for Fitting Distributions. *Journal of Statistical Software*, *64*, 1–34. https://doi.org/10.18637/jss.v064.i04

Fowler, A. E., Stone, E. C., Irwin, R. E., & Adler, L. S. (2020). Sunflower pollen reduces a gut pathogen in worker and queen but not male bumble bees. *Ecological Entomology*, *45*(6), 1318–1326. https://doi.org/10.1111/een.12915

Fox, J., Weisberg, S. (2019). An R Companion to Applied Regression, Third edition. Sage, Thousand Oaks CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Free, J. B. (1957). The food of adult drone honeybees (Apis mellifera). The British Journal of Animal Behaviour, 5(1), 7–11. https://doi.org/10.1016/S0950-5601(57)80038-0

Gerloff, C. U., Ottmer, B. K., & Schmid-Hempel, P. (2003). Effects of inbreeding on immune response and body size in a social insect, Bombus terrestris. Functional Ecology, 17(5), 582–589. https://doi.org/10.1046/j.1365-2435.2003.00769.x

Giacomini, J. J., Adler, L. S., Reading, B. J., & Irwin, R. E. (2023). Differential bumble bee gene expression associated with pathogen infection and pollen diet. *BMC Genomics*, *24*(1), 157. https://doi.org/10.1186/s12864-023-09143-5

Gross, J, Ligges, U. (2015). _nortest: Tests for Normality_. R package version 1.0-4, <https://CRAN.R-project.org/package=nortest>.

Hillyer, J. F. (2016). Insect immunology and hematopoiesis. Developmental and Comparative Immunology, 58, 102–118. https://doi.org/10.1016/j.dci.2015.12.006

Kluyver, T., Ragan-Kelley, B., Pérez, F., Granger, B., Bussonnier, M., Frederic, J., Kelley, K., Hamrick, J., Grout, J., Corlay, S., Ivanov, P., Avila, D., Abdalla, S., Willing, C., & Jupyter development team. (2016). *Jupyter Notebooks – a publishing format for reproducible computational workflows* (F. Loizides & B. Scmidt, Eds.; pp. 87–90). IOS Press. https://doi.org/10.3233/978-1-61499-649-1-87

Mallon, E. B., Loosli, R., & Schmid-Hempel, P. (2003). Specific versus Nonspecific Immune Defense in the Bumblebee, Bombus terrestris L. *Evolution*, *57*(6), 1444–1447.

Microsoft Corporation. (2018). Microsoft Excel. Retrieved from https://office.microsoft.com/excel

Moret, Y., & Schmid-Hempel, P. (2000). Survival for Immunity: The Price of Immune System Activation for Bumblebee Workers. *Science*, *290*(5494), 1166–1168. https://doi.org/10.1126/science.290.5494.1166

Moret, Y., & Schmid-Hempel, P. (2009). Immune responses of bumblebee workers as a function of individual and colony age: Senescence versus plastic adjustment of the immune function. Oikos, 118(3), 371–378. https://doi.org/10.1111/j.1600-0706.2008.17187.x

Pain, J. (1968). Nutrition et développement des organes sexuels adultes. Traité de Biologie de l'Abeille, 1, 410-435.

Pech-May, F., Medina-Medina, L., May-Itzá, W., Paxton, R., & Quezada-Euán, J. J. (2012). Colony pollen reserves affect body size, sperm production and sexual development in males of the stingless bee Melipona beecheii. *Insectes Sociaux*, *59*. https://doi.org/10.1007/s00040-012-0236-8

Pirk, C. W. W., Boodhoo, C., Human, H., & Nicolson, S. W. (2010). The importance of protein type and protein to carbohydrate ratio for survival and ovarian activation of caged honeybees (Apis mellifera scutellata). *Apidologie*, *41*(1), 62–72. https://doi.org/10.1051/apido/2009055

Ponton, F., Wilson, K., Cotter, S. C., Raubenheimer, D., & Simpson, S. J. (2011). Nutritional Immunology: A Multi-Dimensional Approach. *PLoS Pathogens*, *7*(12), e1002223. https://doi.org/10.1371/journal.ppat.1002223

R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Roger, N., Michez, D., Wattiez, R., Sheridan, C., & Vanderplanck, M. (2017). Diet effects on bumblebee health. Journal of Insect Physiology, 96, 128–133. https://doi.org/10.1016/j.jinsphys.2016.11.002

Rolff, J. (2002). Bateman's principle and immunity. *Proceedings of the Royal Society B: Biological Sciences*, *269*(1493), 867–872. https://doi.org/10.1098/rspb.2002.1959

Rosales, C. (2017). Cellular and Molecular Mechanisms of Insect Immunity. In V. D. C. Shields (Ed.), Insect Physiology and Ecology. InTech. https://doi.org/10.5772/67107

Rousseau, A., & Giovenazzo, P. (2016). Optimizing Drone Fertility With Spring Nutritional Supplements to Honey Bee (Hymenoptera: Apidae) Colonies. Journal of Economic Entomology, 109(3), 1009–1014. https://doi.org/10.1093/jee/tow056

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, *9*(7), Article 7. https://doi.org/10.1038/nmeth.2019

Schmid-Hempel, R., & Schmid-Hempel, P. (1998). Colony performance and immunocompetence of a social insect, Bombus terrestris, in poor and variable environments. Functional Ecology, 12(1), 22–30. https://doi.org/10.1046/j.1365-2435.1998.00153.x

Söderhäll, K., & Cerenius, L. (1998). Role of the prophenoloxidase-activating system in invertebrate immunity. Current Opinion in Immunology, 10(1), 23–28. https://doi.org/10.1016/S0952-7915(98)80026-5

Stabler, D., Paoli, P. P., Nicolson, S. W., & Wright, G. A. (2015). Nutrient balancing of the adult worker bumblebee (Bombus terrestris) depends on the dietary source of essential amino acids. *The Journal of Experimental Biology*, *218*(5), 793–802. https://doi.org/10.1242/jeb.114249

Stürup, M., Baer-Imhoof, B., Nash, D. R., Boomsma, J. J., & Baer, B. (2013). When every sperm counts: Factors affecting male fertility in the honeybee Apis mellifera. *Behavioral Ecology*, *24*(5), 1192–1198. https://doi.org/10.1093/beheco/art049

Szolderits, M. J., & Crailsheim, K. (1993). A comparison of pollen consumption and digestion in honeybee (Apis mellifera carnica) drones and workers. *Journal of Insect Physiology*, *39*(10), 877–881. https://doi.org/10.1016/0022-1910(93)90120-G

Tyler, E. R., Adams, S., & Mallon, E. B. (2006). An immune response in the bumblebee, Bombus terrestris leads to increased food consumption. *BMC Physiology*, *6*(1), 6. https://doi.org/10.1186/1472-6793-6-6

Vaudo, A. D., Patch, H. M., Mortensen, D. A., Tooker, J. F., & Grozinger, C. M. (2016a). 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/10.1073/pnas.1606101113

Vaudo, A. D., Stabler, D., Patch, H. M., Tooker, J. F., Grozinger, C. M., & Wright, G. A. (2016b). Bumble bees regulate their intake of essential protein and lipid pollen macronutrients. *Journal of Experimental Biology*, *219*(24), 3962–3970. https://doi.org/10.1242/jeb.140772

Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J,

Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019). "Welcome to the tidyverse." _Journal of Open Source Software_, *4*(43), 1686. doi:10.21105/joss.01686 <https://doi.org/10.21105/joss.01686>.

Wilfert, L., Gadau, J., & Schmid-Hempel, P. (2007). The Genetic Architecture of Immune Defense and Reproduction in Male Bombus Terrestris Bumblebees. *Evolution*, *61*(4), 804–815. https://doi.org/10.1111/j.1558-5646.2007.00079.x

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*, 134. https://doi.org/10.1186/1471-2105-13-134

Zhao, X., & Liu, Y. (2022). Current Knowledge on Bee Innate Immunity Based on Genomics and Transcriptomics. *International Journal of Molecular Sciences*, *23*(22), 14278. https://doi.org/10.3390/ijms232214278

Reflection

Honors has been one of the most rewarding experiences of my undergraduate career. Through my engagement in the honors program, I have become a more rounded individual and scholar. I've taken think tank courses, done an alumni mentorship, and attended many events I never would have otherwise. I have been challenged and had to think critically about topics I never gave much thought to before. I've been exposed to new perspectives and become more open-minded in the process. These experiences have done a great deal to prepare me to be a graduate student, a scientist, and an engaged member of my community beyond what the traditional coursework for my degree was able to do. My biology degree is why I am here in the first place. My coursework and laboratory experiences have given me the content knowledge and experience necessary to be a biologist. However, I feel that the Honors program has elevated this experience immensely. It has required me to think critically, apply concepts from other disciplines, and synthesize both my knowledge and abilities to create a final project, leading to a whole new level of academic mastery.

My capstone project has been the crown jewel of the whole process. My undergraduate career has included many laboratory and research experiences. However, these have been largely guided by professors and TA's. This capstone has required me to take those experiences and apply and expand on what I have learned in a much more independent manner. It has required me to come up with questions on my own, design my own experiments, analyze data, and share the information I learn, with the help of my mentor of course. It has required me to synthesize all of the things I've learned up until this point and put them together to take a project from the very first stages of conception all the way to dissemination of the results. As an aspiring researcher, this has been so incredibly valuable, giving me experience and skills I need to succeed in my

future endeavors. Not to mention the level of accomplishment this is as an undergraduate looking for graduate positions. Specifically, I've learned a great deal about how to perform literature reviews, such as how to read papers and get the relevant information and how to synthesize everything I've read into a cohesive story. Being organized and taking careful notes is essential in this process. Otherwise, you end up doing much more work than you need to. Another thing I learned was about data analysis. I am taking a data science course this semester for my computer science minor. I feel this project complimented that class well. It was fun to take what I've learned there and be able to get my own data to perform analysis on it. It made it all feel actually relevant and worthwhile. Beyond the python I already was learning in class, I had to delve into R as well, which I knew nothing about. That was definitely tricky, but it helped broaden the scope of my project to the disciplines of computer and data science. This was beneficial to widen my experience in different programming languages and techniques. The whole reason I am doing a computer science minor is to give me tools to use for research, and this project has been valuable in fulfilling that goal.

One of the biggest takeaways for me is what it actually means to do science. Science is very much a process that is challenging, frustrating, rewarding, and sometimes disheartening, all at the same time. As one of my coworkers put it, the reason we call it 're-search' is because it never works the first time! I learned the truth of that statement and the value of persistence when the quantitative PCR I was doing failed multiple times. I had to perform 6 optimization tests before I could actually begin the data collection, which also didn't work the first time. Because of time constraints, I still haven't finished that portion of the project, but am planning on doing so after graduation is over. This is the reality of being a scientist though, and it has made what I

was able to accomplish that much more rewarding. The confidence I have in my abilities has increased and I feel more excited for what the future holds.

I've also improved at scientific writing. I've done plenty of writing in my classes, but never anything like this. Producing quality scientific writing is not simple nor intuitive. It is a skill I had to learn, which honestly was a struggle for me at the beginning. There are many things I was not aware of that contribute to clear communication in a scientific article, which my committee had to point out to me. I was also pushed to think in a critical matter about my results. What did they mean and why were they relevant in the context of both the existing literature and my research question? That required a lot of thoughtful effort when writing the final product. My committee helped guide me through this, which was very helpful. These will be extremely valuable skills because no matter what type of science career I end up, thorough interpretation of results and effective communication in writing is so crucial. Beyond that, I feel my capstone has really raised my capabilities above a surface level competence, which I am so grateful for.

Another rewarding aspect of my capstone experience has been the relationship I've developed with my mentor, Dr. Karen Kapheim. Her guidance was crucial to my capstone journey. She was very supportive of my goals and assisted me in developing a project that catered to my interests. This is a relationship I am very grateful for. Having a mentor also prepared me for graduate school and finding a supervisor. The advice I have been given will be so helpful in applying to grad school. Overall, my mentor relationship has been a meaningful and fulfilling part of completing my capstone.

Finally, I believe my capstone project will contribute to our understanding of male bumble bees. It of course won't be a groundbreaking publication, but it does fill a gap in the literature. Males overall are not studied as much as females, and this research could help us

understand how males contribute to the health and success of a colony in the wild. Communication of my results is an important aspect of community engagement as a scientist. I hope to continue talking to people about my project, finding opportunities to present, and work on getting my paper published in a scientific journal. Through this, I hope my capstone experience can have a broader impact on my community, including the bees that live here. This is important for me and those around me, as it provides much of the motivation for why we do research. We want to make a difference in the world, and I think that is important to keep in mind while doing research.

Author Biography

Carson Stoker is graduating with a major in biology and an emphasis in cellular and molecular biology. He also has minors in Computer Science and Chemistry. Carson is a USU Presidential Scholar, Honors student, and Undergraduate Research Scholar. He has been awarded the Gene and Ruth Miller Scholarship, the Dr. Robert Errol Jones Scholarship, the College of Science Dean's Scholarship, and the 'A' Pin Award. Carson has worked in the Kapheim lab for the past three semesters on various bee projects. Under the guidance of Dr. Kapheim, he developed his own research project to study the immune system and influence of diet in male bumble bees. After graduation, Carson is planning on taking a gap year before applying for graduate school to continue studying cellular and molecular biology. He is interested in cancer biology, the immune system, synthetic biology, and of course bees. In his free time, Carson also enjoys reading, camping, hiking, and rock climbing.