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The Effects of Oral and Venereal Transmission of Deformed wing virus on Honey Bee (Apis mellifera) Queen and Colony Health

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THE EFFECTS OF ORAL AND VENEREAL TRANSMISSION OF *DEFORMED WING VIRUS* ON HONEY BEE (*APIS MELLIFERA*) QUEEN AND COLONY HEALTH

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ABSTRACT

Honey bees are important pollinators necessary for the production of many foods. Managed honey bee colonies have been experiencing high levels of colony loss over the last decade due to a combination of different factors. Parasites, pathogens, and queen failure are repeatedly reported as major causes for colony loss, but there is little research exploring the relationship between honey bee viruses and queens. *Deformed wing virus*, a major honey bee virus, has a worldwide distribution, is extremely prevalent, and can infect all castes and life stages. Honey bee queens can be infected with the virus through multiple transmission routes and can be a major source of disease spread within a colony due to close contact with workers in addition to vertical transmission to the eggs she lays. It is important to understand how route of virus transmission impacts the establishment and intensity of virus infection in queens, as well as how this virus exposure could potentially impact colony strength and health. I tested this by artificially inoculating honey bee queens through oral and venereal transmission, assessing virus presence in inoculated queens over time, collecting colony assessments on colonies with inoculated queens, and running virus analysis on brood from inoculated queens. I also tested transgenerational immune priming by challenging pupae of inoculated queens with an injection of DWV-A and recording mortality, symptom development, emergence time, and virus presence and titer. I concluded that single exposure through oral and venereal transmission does not lead to an established high titer infection in queens, and that colonies with these queens are not negatively impacted as far as colony strength parameters or disease development in brood. I also found evidence that transgenerational immune priming may be occurring and expression of it differs based on genotype. Therefore I conclude that single inoculation of queens with DWV-A does not largely negatively impact honey bee queens or the colonies headed by these queens except for cases where the genotype of the queen

is found to be susceptible to DWV-A which could result in queen/colony failure, and that maternal virus experience may benefit offspring challenged by the same virus.

CHAPTER 1. LITERATURE REVIEW

1.1. Global Status of Honey Bees

1.1.1. Honey Bee Biology

Honey bees are an incredibly multifaceted and interesting insect that exhibits many behaviors that are rarely found in other organisms. Honey bees are eusocial insects where individuals participate in cooperative brood care, overlapping generations, and exhibit division of labor (Wilson & Hölldobler, 2005). Division of labor in honey bees is determined by a caste system, where every honey bee colony consists of workers who have many jobs within and out of the nest, drones who mate with queens, and a queen who is responsible for reproduction and colony cohesion through pheromones (Winston, 1991). Workers' duties are age dependent with younger workers performing nest building/cleaning, brood and queen tending, and receiving food resources from older workers, while older workers perform more risky tasks like guarding the colony and foraging for pollen and nectar outside the colony (reviewed in in Winston, 1991).

Honey bees demonstrate exceptional communication and orientation skills that are unmatched even by other social insects. Communication between honey bees involves chemical cues as well as a complex dance language (Winston, 1991). There are over 18 known pheromone signals honey bees utilize across all castes for orientation, kin recognition, inhibition of queen production, raising the alarm for defense, and stimulating worker to perform duties to name a few (reviewed in Winston, 1991). In addition to these chemical cues, honey bees have developed a complex dance language that uses physical body movements to inform other workers of the location for potential food resources and potential nest sites (Winston, 1991). Through this dance language honey bees are able to communicate distance, direction in relation to the position of the sun, and the quality of resources or nest site (Winston, 1991).

1.1.2. Importance of Honey Bees as Pollinators & Colony Loss

Honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae) are important, highly valued pollinators that play significant roles in human systems and in natural systems. Pollination is essential for the production of many of our foods and for the maintenance of wild plants, which allows for increased plant diversity in ecological systems. Most plants rely on animal assisted pollination, which has been shown to improve crop yields and seed production (Klein et al., 2007; Levin, 1983; National Research Council, 2007; Southwick & Southwick Jr, 1992). Although honey bees are not the only available pollinators for crops, native insect pollinator populations are on the decline in the United States and are unable to provide the pollination requirements for large agricultural areas (Kremen et al., 2002; National Research Council, 2007; Potts, Biesmeijer, et al., 2010; Ricketts et al., 2008). Agriculture and food production is expected to increase to meet the demands of the growing global human population. The production of crops that depend on animal pollination has been increasing for the last couple decades, which increases the need for consistent efficient animal pollination (Calderone, 2012). Crop production hasn't yet been negatively impacted by limited pollinator populations, but as the demand for food increases pollinators may not be able to contribute enough pollination services to meet the needs of farmers. This demand for pollination is reflected in the total value attributed to honey bee pollination of these crops, with honey bee pollination of directly dependent crops increasing to $11.68 billion in 2009, and honey bee pollination of indirectly dependent crops totaling $5.39 billion in 2009 (Calderone, 2012). This puts the value of honey bee pollination at $17 billion in 2009. This doesn't account for the other products of the managed honey bee industry such as honey, beeswax, queens, and packages, which has been valued at over $163,100,000 (USDA-NASS, 2018).

While the total number of honey bee colonies has been on the rise globally, honey bees have been experiencing high rates of colony loss in the US and Europe (FAO, 2018). In the United States the total number of managed honey bee colonies has been declining from 1947 to 2010, from 5.9 million colonies to 2.68 million (Calderone, 2012; National Research Council, 2007). Europe also has been experiencing a decline in managed honey bee colonies with a loss of 12% of their managed honey bee colonies from 1961 (21 million) to 2016 (18.5 million) (FAO, 2018; Potts, Roberts, et al., 2010). Colony loss can be attributed to the combined effects of agricultural pesticides, honey bee pests, pathogens, reduced available forage, and poor management practices. One major concern over the declining populations of managed honey bee colonies, is the inability to keep up with pollination demands (Aizen & Harder, 2009). Migratory beekeeping plays an essential role for apiculture and agriculture in America to ensure that crops are sufficiently pollinated. However, the process of migrating over 1 million honey bee colonies across the United States has the potential to cause stress and further exacerbate colony losses due to other health threats. Therefore, colony health and the role of stressors should be further studied.

1.1.3. Honey Bee Health Threats

The combination of multiple factors negatively affects honey bee health including pests, pathogens, agricultural pesticides, lack of available forage, and poor management practices, with pests and pathogens significantly contributing to declines in honey bee health and colony loss. There are numerous pests and pathogens afflicting honey bees, and they often work together synergistically. *Varroa destructor* Anderson & Trueman is a serious concern due to its feeding behavior and capability of vectoring diseases including *Deformed wing virus* (DWV), *Chronic bee paralysis virus* (CBPV), *Acute bee paralysis virus* (ABPV), *Kashmir bee virus* (KBV), *Israeli acute paralysis virus* (IAPV) complex, as well as other diseases that have been associated with

Varroa mite infestation (Bakonyi et al., 2002; Ball, 1983; Ball & Allen, 1988; Bowen-Walker et al., 1999; Celle et al., 2008; Chen et al., 2004; Di Prisco, Pennacchio, et al., 2011). There are over 15 different viruses plaguing honey bees. Of these 15+ viruses, there are 6 common viruses actively being researched due to the serious threats they pose honey bee health: DWV, CBPV, *Black queen cell virus* (BQCV), *Sacbrood virus* (SBV), IAPV, and ABPV. Within these six common honey bee viruses, DWV is the most prevalent (Tentcheva et al., 2004; Traynor et al., 2016). DWV is associated with overwintering colony losses of *Varroa* infested colonies (Dainat et al., 2012; Genersch et al., 2010). This association between the *Varroa* mite and DWV has led to abundant research in an effort to better understand their effects on honey bee health and colony loss.

1.2. *Deformed wing virus*

DWV is a single, positive strand RNA iflavirus from the family Iflaviridae in the order Picornavirales (Lanzi et al., 2006). The structure of the DWV virion and capsid proteins was recently discovered (Škubník et al., 2017). With this discovery, it was noted that the P domain of DWV is a conserved structure among iflaviruses, indicating that these conserved features may be functionally important for the virus's success (Škubník et al., 2017). Škubník et al. (2017) speculates that the function of these features on the P domain may involve receptor or substrate binding sites, or alternatively are used to escape from host endosomes which aids in the virus's ability to circumvent immune response mechanisms (Škubník et al., 2017).

DWV is a quasispecies that consists of multiple master variants, DWV-A, DWV-B, DWV-C (Mordecai, Wilfert, et al., 2016; Mordecai, Brettell, et al., 2016). These variants exhibit different levels of prevalence and different effects on colony health, with DWV-A being the most prevalent and leads to colony loss in the presence of *Varroa* (Di Prisco, Zhang, et al., 2011; Kevill et al.,

2019; Martin et al., 2012; Mordecai, Brettell, et al., 2016). The presence of *Varroa* leads to changes in diversity of DWV, with variant A being selected for over the other variants during *Varroa* transmission of DWV (Martin et al., 2012; Ryabov et al., 2014). However, recent studies have shown variant B to be increasing in prevalence and virulence and is capable of recombination with variant A (Kevill et al., 2019; McMahon et al., 2016; Ryabov et al., 2017).

DWV is a global threat to honey bees due to its worldwide distribution. DWV was first discovered in Japan in colonies infested with *Varroa* and was determined to be the cause of brood and adult mortality (Ball, 1983). DWV was able to achieve a global distribution due human-mediated establishment of *A. mellifera* for beekeeping and agricultural needs around the world (Wilfert et al., 2016). The *Varroa* mite has an important temporal role in the spread of DWV and its now global distribution. Since the *Varroa* mite is capable of increasing DWV prevalence and virulence in honey bees, the virus is able to spread to *Varroa* free colonies through interactions with infected bees (Wilfert et al., 2016). DWV is known to exist at low levels within colonies regardless of the presence of *Varroa*, making it a constant concern for significant disease outbreak should colonies become infested with the mite (Francis et al., 2013).

DWV is a serious threat to honey bee health due to its high prevalence and its worldwide distribution, closely mimicking the distribution of *V. destructor* (a pest that plays a significant role in the transmission of the virus) (Martin & Brettell, 2019). DWV appears to benefit from its relationship with the *Varroa* mite since the mite enables the virus to bypass the bee's natural barriers for viral infections by injecting the virus into the bee through its feeding behavior (Evans & Spivak, 2010; Ongus et al., 2004; Ryabov et al., 2014; Yue & Genersch, 2005). Another benefit of this relationship between the *Varroa* mite and the virus is that the *Varroa* mite even displays immunosuppression capabilities that enables the virus to easily infect the bee (De Miranda &

Genersch, 2010; Di Prisco et al., 2016; Gregory et al., 2005; Yang & Cox-Foster, 2007; Yang & Cox-Foster, 2005). Di Prisco et al. (2016) has proposed a hypothesis that the relationship between DWV and *V. destructor* is mutualistic symbiotic with the virus having a positive effect on *Varroa* feeding and reproduction and the mite vectors the virus (Di Prisco et al., 2016). Di Prisco et al. (2016) also presents evidence that the covert infection of DWV results in immunosuppression by interfering with NF-κB signaling which is not only involved in the Toll pathway but other areas of immune response as well (Di Prisco et al., 2016). Since DWV and the *Varroa* mite are so prolific in honey bee colonies and have a highly synergistic relationship, further research is needed on the virus and the mite to assess health concerns and potential solutions.

DWV can have devastating outcomes on colony health and survival due to its ability to infect multiple life stages. Symptoms of DWV include deformed wings, which is the characteristic symptom of this virus's infection, decreased body size and weight, discoloration of adults, premature pupal death, and a severely reduced adult lifespan (De Miranda & Genersch, 2010). DWV can infect all honey bee castes and life stages, making it a threat for all honey bees (Chen, Evans, et al., 2006; Chen & Siede, 2007). DWV can be transmitted horizontally through honey bee feeding behavior, brood feeding, queen attending, and food processing (worker-worker, worker-queen, worker-drone) and through mating (drone-queen), and can be vertically transmitted during egg laying (queen-offspring) (Chen, Evans, et al., 2006; De Miranda & Fries, 2008; Fievet et al., 2006; Yue et al., 2006; Yue et al., 2007). The other major route of transmission for DWV to workers and drones is through the parasitic relationship with *V. destructor* where the mite acts as a vector, which is discussed in depth earlier (Bowen-Walker et al., 1999). Pathogen virulence has been shown to differ based on route of transmission, with horizontal transmission relying less on host survival and reproduction resulting in higher virulence than vertical transmission which host

survival and reproduction is essential for pathogen transmission and survival (Clayton & Tompkins, 1994). This raises questions about how honey bees are able to survive the constant stress of viruses and *Varroa* infestation, how honey bees manage virus infections, and why some colonies die when others do not.

1.3. Pathogen Defense

1.3.1. Honey Bee Immune Response

Insects differ immensely in immune system function compared with vertebrates, and within insects there is diversity in immune physiology and mechanisms. Insect immune systems lack antibodies and lymphocytes, proteins and cells responsible for immune specificity and immune response in vertebrates (López et al., 2014; Salmela et al., 2015). Despite this, there is evidence that invertebrates can induce an immune response and some cases exhibit specificity (Sadd & Schmid-Hempel, 2006). Social insects, including the honey bee, was found to possess significantly reduced immune response genes than solitary insects (Barribeau et al., 2015; Evans et al., 2006). This is a cause for concern because social insects are prime targets for infection due to interactions between thousands of individuals within a single nest environment with stored resources. However, honey bee colonies are able to thwart disease outbreak with the combined effort of individual defenses through immune genes and group defenses through social and behavioral barriers to disease (Cremer et al., 2017; Evans & Spivak, 2010).

Honey bees utilize physiological immune response as well as social/behavioral techniques to defend against pathogens. Their immune system is expansive, utilizing multiple pathways and processes such as Toll, Imd, JNK, and JAK/STAT for bacterial and fungal infections, and siRNA/RNAi, Toll, Imd, and JAK/STAT for viral infections (Brutscher & Flenniken, 2015; Evans et al., 2006). Honey bees have multiple behaviors that contribute to group defense: hygienic

behavior, antimicrobial nest materials, social "fever", transference of immune traits, and increased risk behavior of infected individuals (Cremer et al., 2017; Evans & Spivak, 2010). In addition to these group defenses, there is a hypothesis that queen polyandry is a possible defense mechanism against parasites and disease by increasing the genetic diversity in offspring (Seeley & Tarpy, 2006; Sherman et al., 1988; Tarpy, 2003). Transference of immune traits is of particular interest because of its ability to bolster the defense of offspring against diseases, which would be especially beneficial for social insects. This process involves either the inoculation of juvenile/newly emerged insects with low doses of pathogens or through transgenerational immune priming where the parental immune experience is passed onto the offspring (Sadd et al., 2005; Traniello et al., 2002).

1.3.2. Transgenerational Immune Priming

Transgenerational immune priming (TgIP) could be a beneficial immune function within social insects but little research has been done on this process in insects especially with viruses. TgIP is defined as enhanced progeny resistance to infection due to parental immune experience of pathogen exposure being passed on to their offspring (López et al., 2014). The exposure of the honey bee queen to parasites and pathogens is a good indication of the types of parasite and pathogen exposure her offspring might encounter, therefore TgIP could be a beneficial adaptation for social insects by giving offspring a head start on immune system development and experience, and ultimately reducing mortality of progeny (Freitak et al., 2009).

Research on TgIP in insects is relatively new. An early study by Little et al. 2003 examined the potential for TgIP by exposing female *Daphnia magna* Straus to *Pasteuria ramosa*, a gram-positive bacterium, and then challenging the females' offspring with *P. ramosa* of either the same strain, or a different strain (Little et al., 2003). They found that challenges of the same strains

reduced overall infectivity in the offspring than that of different strains (Little et al., 2003). TgIP was next demonstrated in *Bombus terrestris* L. where queens challenged by *Arthrobacter globiformis* Conn and Dimmick, a gram-negative bacteria, led to increased antibacterial activity in offspring exposed to the same bacterial challenge (Sadd et al., 2005). A follow-up study found that eggs laid by bacterial challenged *B. terrestris* queens exhibited elevated antibacterial activity demonstrating that the offspring benefit from the mother's immune experience within the egg before hatching, further supporting a change in immune response due to maternal immune experience rather than environmental conditions (Sadd et al., 2005; Sadd & Schmid-Hempel, 2007). These studies have shown that some social insects are capable of TgIP by supplying their offspring with some factor that increases their antimicrobial response to bacterial pathogens, giving the offspring an advantage and potentially increased survivability. TgIP resulting in increased immune response has also been demonstrated for *Tenebrio molitor* L., *Ephestia kuehniella* Zeller, *Trichoplusia ni* Hübner, *Tribolium castaneum* Herbst (Freitak et al., 2009; Moret, 2006; Rahman et al., 2004; Roth et al., 2010). Immune priming has been demonstrated in insects with bacterial infections finding evidence for horizontal immune priming, elevated antimicrobial response, fungal resistance, maternally and paternally derived TgIP, and mechanisms for TgIP in *A. mellifera* (Eggert et al., 2014; Freitak et al., 2014; Hamilton et al., 2010; Knorr et al., 2015; Konrad et al., 2012; Milutinović & Kurtz, 2016; Moret, 2006; Moret & Schmid-Hempel, 2001; Roth et al., 2010; Salmela et al., 2015; Zanchi et al., 2011). However, there is a lack of research on immune priming for viruses.

Few studies have evaluated viral TgIP in insects. Tidbury et al. (2010) tested TgIP in *P. interpunctella* by feeding adults a diet containing a LD_1 virus solution of the DNA virus PiGV (*Plodia interpunctella granulosis virus*) (Tidbury et al., 2010). The adults then mated and offspring

were selected and fed a diet containing a LD_{50} virus solution of PiGV. They found that the offspring of parents that were exposed to PiGV were more resistant to a PiGV challenge (Tidbury et al., 2010). Longdon et al. 2013 tested whether immune priming within *D. melanogaster* was occurring after an initial low dose exposure of *Drosophila c virus* (DCV), a positive sense RNA virus. They found that secondary exposure to DCV resulted in statistically similar levels of mortality between the unexposed individuals and individuals previously exposed to a low dose of DCV, thus that immune priming was not occurring (Longdon et al., 2013). Viral immune priming does not seem to be universal in that some insect-virus systems may allow for TgIP to occur, while other insect-virus systems may not. Each insect-virus system is unique and complex and the mechanisms involved can vary drastically between insect groups, just as insect immune systems vary widely between insect groups (Milutinović & Kurtz, 2016; Tidbury et al., 2010; Vodovar & Saleh, 2012). This is supported by numerous studies conducted on within-generation immune priming in insects with contradicting results about whether or not immune priming is occurring. Viral TgIP has only been tested with viruses in two insect groups, Lepidoptera and Diptera, therefore it would be interesting to see if there is evidence for viral TgIP in Hymenoptera, specifically in eusocial Hymenoptera where it would be beneficial to have the capability to transfer immune protection to offspring.

1.4. The Honey Bee Queen

The honey bee queen has a vital role to play in not only colony dynamics, but also in colony health and survival. The queen is the sole reproductive within a colony, responsible for the production of all male and female offspring. Honey bee queens have two important jobs within the colony: the first is to provide a large number of healthy offspring for colony survival; and the second is to ensure colony cohesion through her pheromones (Slessor et al., 2005). Honey bee

queens are polyandrous, mating with on average more than 10 drones after which sperm travels migrated from the oviducts into the spermatheca and excess sperm is purged (Winston, 1991). Queens on average lay over 175,000 eggs annually over their lifespan which typically lasts under 4 years depending on the amount and viability of the sperm stored in the spermatheca (Winston, 1991). With colony loss being a major concern for beekeepers, one of the suspected leading causes of colony losses is attributed to poor queen quality, which encompasses many different attributes from physical qualities to parasite and pathogen infections (Seitz et al., 2015; vanEngelsdorp et al., 2008). If a queen is deemed unfit by the workers either due to reduced egg production, reduced pheromone production, or poor health, then she will likely be replaced by a supersedure queen (Winston, 1991). Queen replacement can impact the success of the colony because the process puts the colony's population at risk of a significant decline since new brood cannot begin development until a queen is reared, mated, and egg laying begins, which is a process that lasts at least two months (Tarpy et al., 2000). Therefore it is vital that honey bee colonies contain and maintain healthy queens.

1.4.1. Queen Health

Multiple factors impact queen quality and health including physical characteristics, mating success, and infection with parasites and pathogens. Parasites and pathogens play a role in queen health through the direct impact they have on the queen, and through the indirect impact on the rest of the colony. The queen can play a major role in intra-colony disease spread through her interactions with workers and drones, as well as through vertical transmission when a pathogen is passed to her offspring through her eggs. Queens are susceptible to most of the diseases and viruses that plague honey bees, including *N. apis*, ABPV, KBV, IAPV, BQCV, CBPV, DWV, and SBV, as well as the tracheal mite *Acarapis woodi* (Chen et al., 2005; Francis et al., 2013; Villa & Danka,

2005; Webster et al., 2004). Of these possible pathogens, DWV has the highest prevalence in honey bee queens and can be found in multiple tissues throughout the body including the ovaries and spermatheca (Chen, Pettis, et al., 2006;Chen et al., 2005). DWV has been associated with degeneration of ovariole follicles in honey bee queens, however this association did not result in loss of function or fitness in queens (Gauthier et al., 2011). Few studies have been conducted to determine the exact effect that parasites and pathogens have on queen health and performance, but we can speculate that these factors can not only stress the queen and negatively impact her health but also can be easily spread throughout the colony due to the close contact the queen has with her workers as well as through vertical transmission through her eggs.

1.5. Objectives

With colony survival being an important area of interest for honey bee researchers and beekeepers, it is important to try to explain the major factors causing colony losses. Although this is a complex issue with many different stressors affecting honey bees, we have decided to focus our efforts on the impact of DWV due to its worldwide distribution and high prevalence. DWV has a high prevalence in honey bee queens, but little research has been done on determining the impact this viral infection has on the queen and subsequently the colonies headed by infected queens. This research aims to evaluate the effects of DWV exposure to honey bee queens, quantify the effects of DWV exposure to queens on colony strength parameters and colony health, and to identify how the initial infection route of the queens impact the outcome of virus exposure to offspring.

1.6. References

Aizen, M. A., & Harder, L. D. (2009). The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Current Biology, 19*(11), 915-918.

Bakonyi, T., Farkas, R., Szendröi, A., Dobos-Kovács, M., & Rusvai, M. (2002). Detection of acute bee paralysis virus by RT-PCR in honey bee and Varroa destructor field samples: rapid screening of representative Hungarian apiaries. *Apidologie, 33*(1), 63-74.

Ball, B. (1983). *Association of Varroa jacobsoni with virus diseases of honey bees.* Varroa jacobsoni Oud. affecting honey bees: present status and needs: proceedings of a meeting of the EC Experts' Group.

Ball, B., & Allen, M. (1988). The prevalence of pathogens in honey bee (Apis mellifera) colonies infested with the parasitic mite Varroa jacobsoni. *Annals of Applied Biology, 113*(2), 237-244.

Barribeau, S. M., Sadd, B. M., du Plessis, L., Brown, M. J., Buechel, S. D., Cappelle, K., . . . Erler, S. (2015). A depauperate immune repertoire precedes evolution of sociality in bees. *Genome Biology, 16*(1), 83.

Bowen-Walker, P., Martin, S., & Gunn, A. (1999). The transmission of Deformed wing virus between honeybees (Apis mellifera L.) by the ectoparasitic mite Varroa jacobsoni Oud. *Journal of Invertebrate Pathology, 73*(1), 101-106.

Brutscher, L. M., & Flenniken, M. L. (2015). RNAi and antiviral defense in the honey bee. *Journal of Immunology Research, 2015*.

Calderone, N. W. (2012). Insect pollinated crops, insect pollinators and US agriculture: trend analysis of aggregate data for the period 1992–2009. *PLOS One, 7*(5), e37235.

Celle, O., Blanchard, P., Olivier, V., Schurr, F., Cougoule, N., Faucon, J.-P., & Ribière, M. (2008). Detection of Chronic bee paralysis virus (CBPV) genome and its replicative RNA form in various hosts and possible ways of spread. *Virus Research, 133*(2), 280-284.

Chen, Y., Evans, J., & Feldlaufer, M. (2006). Horizontal and vertical transmission of viruses in the honey bee, Apis mellifera. *Journal of Invertebrate Pathology, 92*(3), 152-159.

Chen, Y., Pettis, J. S., Collins, A., & Feldlaufer, M. F. (2006). Prevalence and transmission of honeybee viruses. *Applied and Environmental Microbiology, 72*(1), 606-611.

Chen, Y., Pettis, J. S., Evans, J. D., Kramer, M., & Feldlaufer, M. F. (2004). Transmission of Kashmir bee virus by the ectoparasitic mite Varroa destructor. *Apidologie, 35*(4), 441-448.

Chen, Y., Pettis, J. S., & Feldlaufer, M. F. (2005). Detection of multiple viruses in queens of the honey bee Apis mellifera L. *Journal of Invertebrate Pathology, 90*(2), 118-121.

Chen, Y. P., & Siede, R. (2007). Honey bee viruses. *Advances in Virus Research, 70*, 33-80.

Clayton, D. H., & Tompkins, D. M. (1994). Ectoparasite virulence is linked to mode of transmission. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 256*(1347), 211-217.

Cremer, S., Pull, C. D., & Fürst, M. A. (2017). Social immunity: emergence and evolution of colony-level disease protection. *Annual Review of Entomology*(0).

Dainat, B., Evans, J. D., Chen, Y. P., Gauthier, L., & Neumann, P. (2012). Dead or alive: deformed wing virus and Varroa destructor reduce the life span of winter honeybees. *Applied and Environmental Microbiology, 78*(4), 981-987.

De Miranda, J., & Fries, I. (2008). Venereal and vertical transmission of deformed wing virus in honeybees (Apis mellifera L.). *Journal of Invertebrate Pathology, 98*(2), 184-189.

De Miranda, J. R., & Genersch, E. (2010). Deformed wing virus. *Journal of Invertebrate Pathology, 103*, S48-S61.

Di Prisco, G., Annoscia, D., Margiotta, M., Ferrara, R., Varricchio, P., Zanni, V., . . . Pennacchio, F. (2016). A mutualistic symbiosis between a parasitic mite and a pathogenic virus undermines honey bee immunity and health. *Proceedings of the National Academy of Sciences, 113*(12), 3203-3208.

Di Prisco, G., Pennacchio, F., Caprio, E., Boncristiani Jr, H. F., Evans, J. D., & Chen, Y. (2011). Varroa destructor is an effective vector of Israeli acute paralysis virus in the honeybee, Apis mellifera. *Journal of General Virology, 92*(1), 151-155.

Di Prisco, G., Zhang, X., Pennacchio, F., Caprio, E., Li, J., Evans, J. D., . . . Chen, Y. P. (2011). Dynamics of persistent and acute deformed wing virus infections in honey bees, Apis mellifera. *Viruses, 3*(12), 2425-2441.

Eggert, H., Kurtz, J., & Diddens-de Buhr, M. F. (2014). Different effects of paternal trans-generational immune priming on survival and immunity in step and genetic offspring. *Proceedings of the Royal Society B: Biological Sciences, 281*(1797).

Evans, J., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J. L., Jiang, H., . . . Hultmark, D. (2006). Immune pathways and defence mechanisms in honey bees Apis mellifera. *Insect Molecular Biology, 15*(5), 645-656.

Evans, J. D., & Spivak, M. (2010). Socialized medicine: individual and communal disease barriers in honey bees. *Journal of Invertebrate Pathology, 103*, S62-S72.

FAO. (2018). *FOASTAT*.

Fievet, J., Tentcheva, D., Gauthier, L., De Miranda, J., Cousserans, F., Colin, M. E., & Bergoin, M. (2006). Localization of deformed wing virus infection in queen and drone Apis mellifera L. *Virology Journal, 3*(1), 16.

Francis, R. M., Nielsen, S. L., & Kryger, P. (2013). Patterns of viral infection in honey bee queens. *Journal of General Virology, 94*(3), 668-676.

Freitak, D., Heckel, D. G., & Vogel, H. (2009). Dietary-dependent trans-generational immune priming in an insect herbivore. *Proceedings of the Royal Society of London B: Biological Sciences, 276*(1667), 2617-2624.

Freitak, D., Schmidtberg, H., Dickel, F., Lochnit, G., Vogel, H., & Vilcinskas, A. (2014). The maternal transfer of bacteria can mediate trans-generational immune priming in insects. *Virulence, 5*(4), 547-554.

Gauthier, L., Ravallec, M., Tournaire, M., Cousserans, F., Bergoin, M., Dainat, B., & De Miranda, J. R. (2011). Viruses associated with ovarian degeneration in Apis mellifera L. queens. *PLOS One, 6*(1), e16217.

Genersch, E., Von Der Ohe, W., Kaatz, H., Schroeder, A., Otten, C., Büchler, R., . . . Gisder, S. (2010). The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. *Apidologie, 41*(3), 332-352.

Gregory, P. G., Evans, J. D., Rinderer, T., & De Guzman, L. (2005). Conditional immune-gene suppression of honeybees parasitized by Varroa mites. *Journal of Insect Science, 5*(7), 1-5.

Hamilton, C., Lejeune, B. T., & Rosengaus, R. B. (2010). Trophallaxis and prophylaxis: social immunity in the carpenter ant Camponotus pennsylvanicus. *Biology Letters, 7*(1), 89-92.

Kevill, J. L., de Souza, F. S., Sharples, C., Oliver, R., Schroeder, D. C., & Martin, S. J. (2019). DWV-A lethal to honey bees (Apis mellifera): a colony level survey of DWV variants (A, B, and C) in England, Wales, and 32 states across the US. *Viruses, 11*(5), 426.

Klein, A.-M., Vaissiere, 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 of London B: Biological Sciences, 274*(1608), 303-313.

Knorr, E., Schmidtberg, H., Arslan, D., Bingsohn, L., & Vilcinskas, A. (2015). Translocation of bacteria from the gut to the eggs triggers maternal transgenerational immune priming in Tribolium castaneum. *Biology Letters, 11*(12).

Konrad, M., Vyleta, M. L., Theis, F. J., Stock, M., Tragust, S., Klatt, M., . . . Cremer, S. (2012). Social transfer of pathogenic fungus promotes active immunisation in ant colonies. *PLoS Biology, 10*(4).

Kremen, C., Williams, N. M., & Thorp, R. W. (2002). Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences, 99*(26), 16812-16816.

Lanzi, G., De Miranda, J. R., Boniotti, M. B., Cameron, C. E., Lavazza, A., Capucci, L., . . . Rossi, C. (2006). Molecular and biological characterization of deformed wing virus of honeybees (Apis mellifera L.). *Journal of Virology, 80*(10), 4998-5009.

Levin, M. (1983). Value of bee pollination to US agriculture. *American Entomologist, 29*(4), 50-51.

Little, T. J., O'Connor, B., Colegrave, N., Watt, K., & Read, A. F. (2003). Maternal transfer of strain-specific immunity in an invertebrate. *Current Biology, 13*(6), 489-492.

Longdon, B., Cao, C., Martinez, J., & Jiggins, F. M. (2013). Previous exposure to an RNA virus does not protect against subsequent infection in Drosophila melanogaster. *PLOS One, 8*(9), e73833.

López, J. H., Schuehly, W., Crailsheim, K., & Riessberger-Gallé, U. (2014). Trans-generational immune priming in honeybees. *Proceedings of the Royal Society B, 281*(1785), 20140454.

Martin, S. J., & Brettell, L. E. (2019). Deformed wing virus in honeybees and other insects. *Annual Review of Virology, 6*.

Martin, S. J., Highfield, A. C., Brettell, L., Villalobos, E. M., Budge, G. E., Powell, M., . . . Schroeder, D. C. (2012). Global honey bee viral landscape altered by a parasitic mite. *Science, 336*(6086), 1304-1306.

McMahon, D. P., Natsopoulou, M. E., Doublet, V., Fürst, M., Weging, S., Brown, M. J., . . . Paxton, R. J. (2016). Elevated virulence of an emerging viral genotype as a driver of honeybee loss. *Proceedings of the Royal Society B: Biological Sciences, 283*(1833), 20160811.

Milutinović, B., & Kurtz, J. (2016). *Immune memory in invertebrates.* Seminars in Immunology.

Mordecai, G. J., Brettell, L. E., Martin, S. J., Dixon, D., Jones, I. M., & Schroeder, D. C. (2016). Superinfection exclusion and the long-term survival of honey bees in Varroa-infested colonies. *The ISME Journal, 10*(5), 1182-1191.

Mordecai, G. J., Wilfert, L., Martin, S. J., Jones, I. M., & Schroeder, D. C. (2016). Diversity in a honey bee pathogen: first report of a third master variant of the Deformed wing virus quasispecies. *The ISME Journal, 10*(5), 1264-1273.

Moret, Y. (2006). 'Trans-generational immune priming': specific enhancement of the antimicrobial immune response in the mealworm beetle, Tenebrio molitor. *Proceedings of the Royal Society of London B: Biological Sciences, 273*(1592), 1399-1405.

Moret, Y., & Schmid-Hempel, P. (2001). Entomology: immune defence in bumble-bee offspring. *Nature, 414*(6863), 506.

National Research Council. (2007). *Status of pollinators in North America*: National Academies Press.

Ongus, J. R., Peters, D., Bonmatin, J.-M., Bengsch, E., Vlak, J. M., & van Oers, M. M. (2004). Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite Varroa destructor. *Journal of General Virology, 85*(12), 3747-3755.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution, 25*(6), 345-353.

Potts, S. G., Roberts, S. P., Dean, R., Marris, G., Brown, M. A., Jones, R., . . . Settele, J. (2010). Declines of managed honey bees and beekeepers in Europe. *Journal of Apicultural Research, 49*(1), 15-22.

Rahman, M. M., Roberts, H. L., Sarjan, M., Asgari, S., & Schmidt, O. (2004). Induction and transmission of Bacillus thuringiensis tolerance in the flour moth Ephestia kuehniella. *Proceedings of the National Academy of Sciences, 101*(9), 2696-2699.

Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A., . . . Mayfield, M. M. (2008). Landscape effects on crop pollination services: are there general patterns? *Ecology Letters, 11*(5), 499-515.

Roth, O., Joop, G., Eggert, H., Hilbert, J., Daniel, J., Schmid-Hempel, P., & Kurtz, J. (2010). Paternally derived immune priming for offspring in the red flour beetle, Tribolium castaneum. *Journal of Animal Ecology, 79*(2), 403-413.

Ryabov, E. V., Childers, A. K., Chen, Y., Madella, S., Nessa, A., & Evans, J. D. (2017). Recent spread of Varroa destructor virus-1, a honey bee pathogen, in the United States. *Scientific Reports, 7*(1), 17447.

Ryabov, E. V., Wood, G. R., Fannon, J. M., Moore, J. D., Bull, J. C., Chandler, D., . . . Evans, D. J. (2014). A virulent strain of Deformed wing virus (DWV) of honeybees (Apis mellifera) prevails after Varroa destructor-mediated, or in vitro, transmission. *PLoS Pathogens, 10*(6), e1004230.

Sadd, B. M., Kleinlogel, Y., Schmid-Hempel, R., & Schmid-Hempel, P. (2005). Trans-generational immune priming in a social insect. *Biology Letters, 1*(4), 386-388.

Sadd, B. M., & Schmid-Hempel, P. (2006). Insect immunity shows specificity in protection upon secondary pathogen exposure. *Current Biology, 16*(12), 1206-1210.

Sadd, B. M., & Schmid-Hempel, P. (2007). Facultative but persistent trans-generational immunity via the mother's eggs in bumblebees. *Current Biology, 17*(24), R1046-R1047.

Salmela, H., Amdam, G. V., & Freitak, D. (2015). Transfer of immunity from mother to offspring is mediated via egg-yolk protein vitellogenin. *PLoS Pathogens, 11*(7), e1005015.

Seeley, T. D., & Tarpy, D. R. (2006). Queen promiscuity lowers disease within honeybee colonies. *Proceedings of the Royal Society B: Biological Sciences, 274*(1606), 67-72.

Seitz, N., Traynor, K. S., Steinhauer, N., Rennich, K., Wilson, M. E., Ellis, J. D., . . . Caron, D. M. (2015). A national survey of managed honey bee 2014–2015 annual colony losses in the USA. *Journal of Apicultural Research, 54*(4), 292-304.

Sherman, P. W., Seeley, T. D., & Reeve, H. K. (1988). Parasites, pathogens, and polyandry in social Hymenoptera. *The American Naturalist, 131*(4), 602-610.

Škubník, K., Nováček, J., Füzik, T., Přidal, A., Paxton, R. J., & Plevka, P. (2017). Structure of deformed wing virus, a major honey bee pathogen. *Proceedings of the National Academy of Sciences, 114*(12), 3210-3215.

Slessor, K. N., Winston, M. L., & Le Conte, Y. (2005). Pheromone communication in the honeybee (Apis mellifera L.). *Journal of Chemical Ecology, 31*(11), 2731-2745.

Southwick, E. E., & Southwick Jr, L. (1992). Estimating the economic value of honey bees (Hymenoptera: Apidae) as agricultural pollinators in the United States. *Journal of Economic Entomology, 85*(3), 621-633.

Tarpy, D. R. (2003). Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. *Proceedings of the Royal Society of London B: Biological Sciences, 270*(1510), 99-103.

Tarpy, D. R., Hatch, S., & Fletcher, D. J. (2000). The influence of queen age and quality during queen replacement in honeybee colonies. *Animal Behaviour, 59*(1), 97-101.

Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M. E., & Bergoin, M. (2004). Prevalence and seasonal variations of six bee viruses in Apis mellifera L. and Varroa destructor mite populations in France. *Applied and Environmental Microbiology, 70*(12), 7185-7191.

Tidbury, H. J., Pedersen, A. B., & Boots, M. (2010). Within and transgenerational immune priming in an insect to a DNA virus. *Proceedings of the Royal Society B: Biological Sciences, 278*(1707), 871-876.

Traniello, J. F., Rosengaus, R. B., & Savoie, K. (2002). The development of immunity in a social insect: evidence for the group facilitation of disease resistance. *Proceedings of the National Academy of Sciences, 99*(10), 6838-6842.

Traynor, K. S., Rennich, K., Forsgren, E., Rose, R., Pettis, J., Kunkel, G., . . . Lopez, D. (2016). Multiyear survey targeting disease incidence in US honey bees. *Apidologie, 47*(3), 325-347.

USDA-NASS. (2018). Annual Honey Report. *National Agricultural Statistics Service*.

vanEngelsdorp, D., Hayes Jr, J., Underwood, R. M., & Pettis, J. (2008). A survey of honey bee colony losses in the US, fall 2007 to spring 2008. *PLOS One, 3*(12), e4071.

Villa, J. D., & Danka, R. G. (2005). Caste, sex and strain of honey bees (Apis mellifera) affect infestation with tracheal mites (Acarapis woodi). *Experimental & Applied Acarology, 37*(3-4), 157.

Vodovar, N., & Saleh, M.-C. (2012). Of insects and viruses: the role of small RNAs in insect defence *Advances in Insect Physiology* (Vol. 42, pp. 1-36): Elsevier.

Webster, T. C., Pomper, K. W., Hunt, G., Thacker, E. M., & Jones, S. C. (2004). Nosema apis infection in worker and queen Apis mellifera. *Apidologie, 35*(1), 49-54.

Wilfert, L., Long, G., Leggett, H., Schmid-Hempel, P., Butlin, R., Martin, S., & Boots, M. (2016). Deformed wing virus is a recent global epidemic in honeybees driven by Varroa mites. *Science, 351*(6273), 594-597.

Wilson, E. O., & Hölldobler, B. (2005). Eusociality: origin and consequences. *Proceedings of the National Academy of Sciences, 102*(38), 13367-13371.

Winston, M. L. (1991). *The Biology of the Honey Bee*: Harvard University Press.

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.

Yang, X., & Cox-Foster, D. L. (2005). Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host immunosuppression and viral amplification. *Proceedings of the National Academy of Sciences of the United States of America, 102*(21), 7470-7475.

Yue, C., & Genersch, E. (2005). RT-PCR analysis of Deformed wing virus in honeybees (Apis mellifera) and mites (Varroa destructor). *Journal of General Virology, 86*(12), 3419-3424.

Yue, C., Schröder, M., Bienefeld, K., & Genersch, E. (2006). Detection of viral sequences in semen of honeybees (Apis mellifera): evidence for vertical transmission of viruses through drones. *Journal of Invertebrate Pathology, 92*(2), 105-108.

Yue, C., Schröder, M., Gisder, S., & Genersch, E. (2007). Vertical-transmission routes for deformed wing virus of honeybees (Apis mellifera). *Journal of General Virology, 88*(8), 2329-2336.

Zanchi, C., Troussard, J. P., Martinaud, G., Moreau, J., & Moret, Y. (2011). Differential expression and costs between maternally and paternally derived immune priming for offspring in an insect. *Journal of Animal Ecology, 80*(6), 1174-1183.

CHAPTER 2. QUEEN INOCULATION & COLONY HEALTH

2.1. Introduction

Honey bee (*Apis mellifera* L.) colonies in the United States have been facing high annual losses in recent years, and RNA viruses have been considered a significant contributor to these losses and overall honey bee health (Ellis et al., 2010; Seitz et al., 2015). There are over 15 recognized viruses plaguing honey bees, the most common being *Deformed wing virus* (DWV), *Black queen cell virus* (BQCV), *Sacbrood virus* (SBV), *Kashmir bee virus* (KBV), *Acute bee paralysis virus* (ABPV), *Israeli acute paralysis virus* (IAPV) and *Chronic bee paralysis virus* (CBPV). Within these six common honey bee viruses, DWV is the most prevalent for workers and queens (Francis et al., 2013; Tentcheva et al., 2004; Traynor et al., 2016). DWV is not only capable of infecting honey bees at all life stages and all castes, but it can also be transmitted horizontally through honey bee feeding behavior (worker-worker, worker-drone, worker-queen) and through mating (drone-queen), and vertically during egg laying (queen-offspring) (Chen, Evans, et al., 2006; De Miranda & Fries, 2008). However, previous research conducted has mainly focused on vector-borne transmission through the parasitic mite *Varroa destructor* (Wilfert et al., 2016). Research has shown differential symptom development based on these different routes of DWV transmission for workers, where pupae injected with the virus to mimic *Varroa* transmission consistently displayed wing deformities (Möckel et al., 2011). This raises questions about whether queens also display differential infection rate and intensity based on the route of transmission.

Queens are susceptible to most of the parasites and pathogens that plague honey bees, including *Nosema apis* Zander, ABPV, KBV, *Israeli acute paralysis virus* (IAPV), BQCV, CBPV, DWV, and SBV, as well as the tracheal mite *Acarapis woodi* (Chen et al., 2005; Francis et al., 2013; Villa & Danka, 2005; Webster et al., 2004). Of these possible pathogens, DWV has the

highest prevalence in honey bee queens and can be found in multiple tissues throughout the body including the ovaries and spermatheca (Chen, Pettis, et al., 2006; Chen et al., 2005; Gauthier et al., 2011). While there have been few studies to determine the effect that pathogens have on queen health and performance, little is known about how transmission route in queens effects dissemination and health within an entire colony.

One of the pathways DWV is spread throughout a honey bee colony is through trophallaxis from worker bees providing food to queens, drones, other workers, and larvae. Trophallaxis could be an important mode of transmission considering that DWV has been found in honey bee food stores, larval food in cells, and worker bee guts and heads (Chen, Evans, et al., 2006; Möckel et al., 2011; Yue & Genersch, 2005). Previous research has shown that inoculating adult workers through oral transmission of DWV results in infection at a significant viral titer (Möckel et al., 2011). Therefore, the role of oral transmission should be further evaluated as a potential significant route of DWV infection for queens.

Another significant mode of DWV transmission in honey bee queens is through mating and egg laying. Queens exhibit polyandry, meaning females mate with more than one male. Therefore, queens have a higher likelihood of being exposed multiple times during mating (Amiri et al., 2016; Amiri et al., 2017; Gauthier et al., 2011). In addition, venereal infection may increase the likelihood of vertical transmission to offspring since DWV has been found in the spermathecae and ovaries of queens following mating with DWV infected drones (Amiri et al., 2016; Chen, Evans, et al., 2006; De Miranda & Fries, 2008).

The goal of this study was to evaluate the impacts of deformed wing virus transmission on honey bee queen health. This was accomplished through two objectives (1) to evaluate the virus levels over time in orally and venereally transmitted DWV to honey bee queens, and (2) to

compare colony strength metrics (adult population, brood, pollen, and nectar) and colony survival for colonies with queens inoculated with DWV through different routes of transmission. In addition, we sampled eggs and pupae of experimentally inoculated queens for viral presence and abundance of four common honey bee viruses (DWV-A, DWV-B, BQCV, and CBPV), which were detected in the queens at the start of the experiment.

2.2. Materials and Methods

2.2.1. Insects

Honey bee queens were reared and maintained at the USDA ARS Honey Bee Breeding, Genetics, and Physiology research lab in Baton Rouge, LA following standard queen rearing procedures (Büchler et al., 2013). Queens were grafted (moving first instar larvae from a brood frame into a queen cell cup) from source colonies headed by Italian queens. Queen cell cups were then placed into queenless colonies where worker bees maintained and reared the queens. Once queen cells were capped, they were moved into individual tubes and maintained in an incubator at 33°C until emergence. Following emergence, queens were placed into chambers within a wooden board and stored in a queenless colony between frames with brood. Queens were stored in the colony for 1-2 weeks and then received treatments, as this is the typical time when queens would naturally mate or be inseminated (Cobey et al., 2013). Queens in objective 1 were grafted from a single source colony headed by an Italian queen, while queens in objective 2 were grafted from three different source colonies headed by Italian queens of different lineages, Italian 202, Italian 205, and Italian 209. Queen sources could be closely or distantly related due to close proximity between the colonies which could result in breeding between colonies. All queens reared from the same source are considered sister queens.

2.2.2. Viral Homogenate Synthesis

DWV symptomatic adult honey bees that were injected with DWV at 10^5 viral titer as pupae and then stored at -80°C upon emergence were used for the viral inoculum for queen treatments. For objective 1, bees were thoroughly ground by hand with a pestle in a frozen state and 1500µL of sterile 1X PBS was added while continuing to hand grind. The samples were then centrifuged in an Eppendorf Centrifuge 5430 R (Hamburg, Germany) at 4°C for 10 minutes at 10,000rpm. The supernatant was then pipetted into 3mL syringes fitted with 0.22µm filters and the supernatant was filter sterilized. Objective 2 followed the same homogenization procedure with the following modifications, bees were hand ground with 1.0mL of sterile 1X PBS and supernatant was centrifuged at 4°C for 20 minutes at 14,000RPM. RNA extraction followed the Promega (Madison, WI) Maxwell® 16 LEV simplyRNA Tissue protocol following the manufacturer's technical manual (Promega, 2017). Upon completion of RNA extraction, the samples were stored at -80°C prior to RNA quantification using Thermo Fisher Scientific (Waltham, MA) NanoDrop One.

cDNA synthesis began by thawing the RNA previously stored at -80°C, and quantifying each sample's ng/µL concentration via NanoDrop One. For objective 1, the Invitrogen™ (Carlsbad, CA) SuperScript™ VILO™ cDNA Synthesis kit and protocol was used. cDNA Master Mix was created for 30µL reactions using 500ng/µL of input RNA. Objective 2 used the same kit and protocol but for 60µL reactions using 100.0ng/µL of input RNA. cDNA synthesis occurred in 0.2mL PCR strip tubes, one tube per sample, and cDNA synthesis was completed using a BioRad (Hercules, CA) T100 thermal cycler (Table 2.1). Once cDNA synthesis was completed, cDNA was quantified using NanoDrop One and the total copies per µL of sample was calculated to verify

the cDNA concentration was uniform across all samples. cDNA was stored at 4°C prior to qPCR standard curve confirmation of copy number.

Table 2.1. cDNA synthesis thermal cycler protocol for two different cDNA synthesis kits: Invitrogen™ SuperScript™ VILO™ and Qiagen QuantiTect® Reverse.

cDNA Synthesis Kit		Step 1	Step 2	Step 3	Step 4	
Invitrogen™ SuperScript™ VILO™ cDNA Synthesis	Temperature	25°C	42°C	85°C	4°C	
	Duration	10 minutes	1 hour 30 minutes	5 minutes	Infinite hold	
Qiagen QuantiTect® Reverse Transcription Kit		Genomic DNA Elimination		Reverse-Transcription		
		Step 1	Step 2	Step 1	Step 2	Step 3
	Temperature	42°C	4°C	42°C	95°C	4°C
	Duration	2 minutes	Infinite hold	25 minutes	3 minutes	Infinite hold

qPCR for viral inoculum synthesis and screening for objective 1 was performed on the BioRad CFX Connect using BioRad SsoAdvanced™ Universal SYBR® Green Supermix. Samples were tested for DWV-A, BQCV, AKI, CBPV, LSV-U, and DWV-B, using standard primer pairs (Table 2.2). Reactions contained 5.0µL master mix, 3.0µL nuclease-free H_2O, 1.0µL cDNA template, and 0.5µL forward and reverse primers for DWV-B, CBPV, AKI, and LSV-U, or 0.37µL forward primer and 0.63µL reverse primer for DWV-A and BQCV. BioRad CFX Connect thermal cycling protocol: 5 min at 95°C, followed by 40 cycles of 95°C for 5s, 53.5°C for 10s for DWV-A and CBPV or 59.0°C for 10s for DWV-B, BQCV, AKI, and LSV-U, 72.0°C for 10s, and melt curve analysis for 65.0°C to 95.0°C for 5s. DWV-A quantification in objective 1 was performed on the ABI (Foster City, CA) QuantStudio6 using Applied Biosystems™ PowerUp™ SYBR™ Green master mix. Reactions contained 10.0µL master mix, 6.0µL nuclease-free H_2O, 2.0µL cDNA template, and 0.75µL forward primer 1.25µL reverse primer. ABI QuantStudio6 thermal cycling protocol: 50.0°C for 20s, 95.0°C for 5 min, followed by 40 cycles of 95.0°C for

5s, 53.5°C for 20s, 72.0°C for 15s, and melt curve analysis for 95.0°C for 15s, 65.0°C for 15s, 95.0°C for 15s. DWV-A quantification based off standard curve comparison where the standard curve was created from linearized plasmid standards ranging from 10^{12} to 10^{5} copies per reaction following standard practices (de Guzman et al., 2017; Hou et al., 2010). The DWV inoculum was identified via real-time qPCR as being predominantly of DWV strain A, the most prevalent and virulent DWV variant (Kevill et al., 2019). The sample with the highest DWV-A titer containing little to no presence of the other viruses was selected to be used for treatments. The chosen sample was split and diluted into working stocks at 10^{10}, 10^{7}, and 10^{4} viral titer level.

qPCR for viral inoculum synthesis and screening for objective 2 was performed on the Applied Biosystems™ QuantStudio™ 6 Flex Real-Time PCR System with the SYBR™ Green PCR Master Mix with the same targets as objective 1. Reactions contained 10.0µL master mix, 6.0µL nuclease-free H_2O, 2.0µL cDNA template, and 1.0µL forward and reverse primers for DWV-B, CBPV, LSV-U and AKI or 0.75µL forward primer 1.25µL reverse primer for DWV-A and BQCV. ABI QuantStudio6 thermal cycling protocol for DWV-A and CBPV: 50.0°C for 20s, 95.0°C for 5 min, followed by 40 cycles of 95.0°C for 5s, 53.5°C for 20s, 72.0°C for 15s, and melt curve analysis for 95.0°C for 15s, 60.0°C for 15s, 95.0°C for 15s. ABI QuantStudio6 thermal cycling protocol for DWV-B, LSV-U, AKI, and BQCV: 95.0°C for 5 min, followed by 40 cycles of 95.0°C for 5s, 59.0°C for 20s, 72.0°C for 10s, and melt curve analysis for 95.0°C for 15s, 65.0°C for 15s, 95.0°C for 15s. Sample selection was the same as for objective 1.

Table 2.2. Primer pairs used for molecular analyses including sequences and references.

Target	Forward Primer	Reverse Primer	Reference
Acute bee paralysis virus, Kashmir bee virus, Israeli acute paralysis virus Complex (AKI)	CTT TCA TGA TGT GGA AAC TCC	AAA CTG AAT AAT ACT GTG CGT A	(Francis & Kryger, 2012)
Black queen cell virus (BQCV)	TCG CAG AGT TCC AAA TAC CG	TAT CAT CTC CCG CAC CAA CC	(de Guzman et al., 2019; Yoo et al., 2008)
Chronic bee paralysis virus (CBPV)	CGC AAG TAC GCC TTG ATA AAG AAC	ACT ACT AGA AAC TCG TCG CTT CG	(Blanchard et al., 2007)
Deformed wing virus-1 (DWV-A)	GAG ATT GAA GCG CAT GAA CA	TGA ATT CAG TGT CGC CCA TA	(Lanzi et al., 2006)
Lake Sinai virus (LSV-U)	CGT GCG GAC CTC ATT TCT TCA TGT	CTG CGA AGC ACT AAA GCG TT	(Daughenbaugh et al., 2015)
Varroa destructor virus -1 (DWV-B)	CTG TAG TTA AGC GGT TAT TAG AA	GGT GCT TCT GGA ATA GCG GAA	(Ryabov et al., 2017)

2.2.3. Queen Treatments

In objective 1, queen treatments consisted of a negative control, positive control, oral inoculation, venereal inoculation, and oral plus venereal inoculation. In all treatment types (except for the negative control) queens received doses of DWV at 10^{10} to mimic high titer infection found in queens and workers, often resulting in overt deformed wing symptoms in workers (Amiri et al., 2016; Gisder et al., 2009). Orally inoculated queens were fed a total volume of 10µL of a 50% sugar water solution mixed with DWV-A virus stock to achieve a 10^{10} viral titer. These queens were inseminated with 3µL of semen and 1µL of insemination buffer for a total volume of 4µL. Insemination buffer was made by combining 400mL of sterilized water, 5.0g of NaCl, and 1.0g of dihydrostreptomycin sesquisulfate (Sigma-Aldrich St. Louis, MO). Venereally inoculated queens were fed 10µL of 50% sugar water diluted with MilliQ water, and inseminated with 3µL of semen mixed with DWV-A virus stock to achieve a 10^{10} viral titer level and 1 µL of insemination buffer.

The combined orally and venereally inoculated queens were fed a total volume of 10µL of a 50% sugar water solution mixed with DWV-A virus to achieve 10^{10} viral titer level within the mixture, and inseminated with 3µL of semen mixed with DWV-A virus stock to achieve a 10^{10} viral titer level and 1 µL of insemination buffer. Negative control queens were fed 10µL of 50% sugar water diluted with MilliQ water and were inseminated with 3µL of semen and 1 µL of insemination buffer for a total volume of 4µL. Positive control queens received the same feeding and insemination regimen as negative controls, but were injected on the dorsal side of the abdomen with 3µL of DWV-A diluted with PBS to achieve a viral titer level of 10^{10} (De Miranda et al., 2013; Gisder et al., 2009; Möckel et al., 2011).

Each queen was inseminated following standard methods, using a homogenized mixture of semen from over 600 drones from low *Varroa* mite colonies (Cobey et al., 2013). CO_2 was used to anesthetize the queens during insemination. Once the queens regained consciousness they were hand fed using a 10µL Rainin (Oakland, CA) single channel pipette. To improve the likelihood of proper feeding, the pipette tip was used to extend the queens' proboscis and the food was deposited on the extended proboscis and in the oral cavity, which the queen then visibly ingested. After treatment, all queens were stored in a queenless colony between frames containing brood on a wooden board with individual chambers for each queen.

Queens in objective 2 received the same treatment methods as objective 1 with the following modifications. Queens were divided into three treatment groups: negative control, oral inoculated, and venereal inoculated. Within each treatment, 30 queens were treated and were split among the three queen sources. Queens receiving virus inoculation received doses of DWV at 10^{7} to mimic moderate infection level since this virus level has been found in queens and drone semen, as opposed to high infection level (10^{10}) which may result in premature queen death (Amiri et al.,

2016). All queens were fed a total volume of 5µL, and inseminated with 5µL of semen and 1 µL of insemination buffer for a total volume of 6µL. Venereal inoculation with DWV was achieved through insemination buffer mixed with DWV, rather than semen mixed with DWV. The semen used for insemination was collected from 1,200 honey bee drones from colonies of Saskatraz honey bees.

2.2.4. Objective 1: Queen DWV-A Infection via Different Transmission Routes

After receiving their treatments, queens were stored within a queenless colony to be maintained by worker honey bees for the duration of the experiment. Queens were sampled at the following time points after treatment: 1 hour, 3 days, 7 days, and 14 days. Sampled queens were taken directly from the colony and placed into -80°C storage. For molecular analysis, queens were removed from -80°C storage and placed into -20°C chilled Omni International 2mL microtubes pre-filled with 1.4mm ceramic beads. Queens were milled using an Omni (Kennesaw, GA) BeadRupter-24 Elite with a protocol of 2 cycles at 5m/s for 25s with a 10s dwell between cycles. Next, 250µL of Promega (Madison, WI) homogenization solution was added to each sample and vortexed upside down for 15 seconds. Then 250µL of Promega (Madison, WI) lysis buffer was added to each sample and vortexed right side up for 15 seconds. Samples were then centrifuged in an Eppendorf 5430R centrifuge for 30 seconds at 10,000RPM at 4°C and stored at 4°C for 10 minutes, and RNA extraction of the homogenate followed the Maxwell® 16 LEV simplyRNA Tissue protocol as mentioned in section 2.2.2 using 400µL of sample homogenate. Upon completion of RNA extraction, the samples were stored at -80°C prior to NanoDrop One quantification. cDNA synthesis was completed as described in section 2.2.2 modified by using the Qiagen (Hilden, Germany) QuantiTect® Reverse Transcription kit and protocol for 40µL reactions using 500.0ng/µL of input RNA (table 2.1). qPCR was conducted following the methods described

in section 2.2.2 for the Applied Biosystems™ QuantStudio™ 6 Flex Real-Time PCR System with the following targets: DWV-A, DWV-B, CBPV, and BQCV.

2.2.5. Objective 2: Colony Health Following Queen Inoculation

2.2.5.1.Experimental Sites

This study was conducted from May 7th, 2018 through September 28th, 2018. These months were selected in accordance with typical honey bee active foraging period throughout the year, avoiding the fall and winter months and focusing on the time of year honey bee queens are most active. Experimental sites were owned and maintained by the USDA ARS Honey Bee Breeding, Genetics, and Physiology research lab in Baton Rouge, LA. The experiment was conducted across two distinct apiaries near St. Gabriel, LA located at 30.320336, -91.092870 and 30.260376, -91.071149. Each apiary began the experiment with colonies from each treatment group and had representation from all three honey bee queen sources to ensure that the different experimental sites would not bias a single treatment or a single queen source. Each colony started off in 8-frame deep boxes with one feeder, one frame of drawn comb, and five frames of foundation.

2.2.5.2.Colony Setup

Bee packages were transported from Bemis Honey Bee Farms in Little Rock, Arkansas to the USDA-ARS Honey Bee Breeding, Genetics, and Physiology research lab in Baton Rouge, Louisiana. The queens reared and treated for this project were then introduced to the packages by placing them into Mann Lake (Marshall, TX) JZBZ cages and were hung within the packages overnight. The following day the packages were introduced to the colonies. Prior to colony introduction, the colonies were set up so that each colony consisted of a single 8-frame box with a feeder, 5 foundation frames, a single frame with drawn out comb, and entrance reducers. Packages and queens were installed into the colonies and checked four days later to ensure the queens were

released from push-in cages and egg laying had begun. If queens had not begun laying eggs, they were treated with a second dose of CO_2 exposure to stimulate egg laying (n=11). A second egg laying check was completed four days later to ensure that the queens who received a second CO_2 dose had begun laying eggs. Queens that were not laying eggs were removed from the experiment (n=1). Brood was checked 15 days after egg laying to determine if insemination was successful by observing if the brood being reared was worker brood or drone brood. Varroa mite treatment (HopGuard II) was given to colonies 1 week after introduction to eliminate any low level infestation.

2.2.5.3.Colony Assessments

Colony assessments were conducted every 6 weeks on all colonies. Colony assessments follow previously established protocols by locating the queen, estimating frame coverage of brood, pollen, and nectar for all frames within the colony, recording estimated total bee population quantified by complete coverage of all frames within the colony, and any notes about the colony (brood pattern, queen cells, pest observations, disease symptoms, etc.) (Delaplane et al., 2013). Eggs and pupae were sampled during colony assessments for viral analysis. Of the eggs and pupae sampled, a targeted subset were chosen for viral analysis. The targeted subset consisted of colonies that experienced queen/colony failure by the end of the field season in September to see if virus presence/infection in the brood contributed to the failure of the colony. In addition to these data collected, a sample of ~300 nurse bees were taken and put on ice to perform *Varroa* mite washes to quantify the number of *Varroa* mites per 100 bees using a modified mite wash protocol from Dietemann et al 2013 (Dietemann et al., 2013; Rinkevich et al., 2017). Mite washes consisted of placing the frozen bees in a plastic cup with a mesh bottom nested in an unaltered plastic cup, the bees were then covered with soapy water and placed on a shaker table for an hour. After an hour

the mesh-bottomed cup was removed and the number of mites in the soapy water was counted. This was repeated until two consecutive zero counts were obtained. Mite washes allowed us to keep an eye on the *Varroa* level within colonies and make decisions about treating for *Varroa* before the colonies became overwhelmed and before the pest would have a chance to vector viruses and impact the disease study.

2.2.5.4.Queen Tissue Viral Analysis

Queens that survived the field season were collected and stored at -80°C for viral analyses. Queens were removed from storage and dissected, separating the gut, spermatheca, ovaries, and rest of body. Tissues were placed into separate tubes and returned to -80°C storage until molecular analysis. Tissue samples were placed into -20°C chilled Omni International 2mL microtubes with 1.4mm ceramic beads and were milled using an Omni BeadRupter-24 Elite with a protocol of 1 cycle at 5m/s for 30s. Body samples received 150µL of Promega homogenization solution and 150µL of Promega lysis buffer and were vortexed inverted for 10 seconds and then right side up for 10 seconds. Body samples were then centrifuged in an Eppendorf 5430R centrifuge for 30 seconds at 10,000RPM at 4°C. Gut, ovaries, and spermatheca received 150µL of Promega homogenization solution and 150µL of Promega lysis buffer. Spermatheca were gently hand ground with a pestle to rupture the tissue. Gut, ovaries, and spermatheca samples were vortexed for 1 minute. RNA extraction of the homogenate followed the Maxwell® 16 LEV simplyRNA Tissue protocol as mentioned in section 2.2.2 using 400µL of sample homogenate. Upon completion of RNA extraction, the samples were stored at -80°C prior to NanoDrop One quantification. cDNA synthesis was completed as described in section 2.2.2 modified by using the Qiagen QuantiTect® Reverse Transcription kit and protocol for 30µL reactions using 250.0ng/µL of input RNA, except for spermathecae, which used 140.0 ng/µL due to lower RNA yields (table

2.1). qPCR was conducted following the methods described in section 2.2.2 for the Applied Biosystems™ QuantStudio™ 6 Flex Real-Time PCR System with the following targets: DWV-A, DWV-B, CBPV, and BQCV.

2.2.5.5.Virus Prevalence in Eggs and Pupae

Each colony selected for analysis (n=14 colonies per treatment) had 10 eggs and pupae sampled and stored at -80°C, 3 of which were randomly selected for molecular analysis. Each egg was placed in the bottom of a 1.5mL Eppendorf tube and 25µL of Promega homogenization solution was added to each tube. The samples were placed in a -20°C freezer for 5 minutes to freeze the homogenization solution and rupture the egg. Samples were removed from the freezer and gently hand ground with a pestle and 25µL of Promega lysis buffer was added to each tube. The samples were vortexed for 10 seconds and 100µL of Promega homogenization solution and 150µL of 1X sterile PBS were added to each tube and vortexed for an additional 10 seconds. Samples were centrifuged at 5,500 RPM for 15 seconds. Pupae were removed from -80°C storage and 250µL of Promega homogenization solution and 250µL of Promega lysis buffer were added to each sample. Samples were hand ground with a pestle, and then centrifuged in an Eppendorf 5430R centrifuge for 60 seconds at 14,000RPM at 4°C. RNA extraction of the homogenate followed the Maxwell® 16 LEV simplyRNA Tissue protocol as mentioned in section 2.2.2 using 400µL of sample homogenate. Upon completion of RNA extraction, the samples were stored at -80°C prior to NanoDrop One quantification. cDNA synthesis was completed as described in section 2.2.2 modified by using the Qiagen QuantiTect® Reverse Transcription kit and protocol for 30µL reactions using 30.0ng/µL of input RNA. qPCR was conducted following the methods described in section 2.2.2 for the Applied Biosystems™ QuantStudio™ 6 Flex Real-Time PCR System with the following targets: DWV-A, DWV-B, CBPV, and BQCV.

2.2.6. Statistical Analysis

2.2.6.1.Objective 1

All statistical analyses were performed in JMP® Pro 14 (SAS Institute Inc, 2019). Each virus transmission route was tested for four common honey bee viruses to see if the inoculation of DWV-A impacted the presence and virus titer of these viruses at each time point after inoculation. Detection of DWV-A, DWV-B, CBPV, and BQCV was analyzed with logistic regression using generalized linear models with binomial distribution where the model effects were time post-inoculation, inoculation route, and time post-inoculation*inoculation route. Post-hoc analyses of presence and absence consisted of categorical response analysis and response homogeneity tests with Pearson's chi-square and Fisher's exact.

Virus titer was corrected by subtracting the average virus titer in non-inoculated queens at 1 hour from all other inoculation treatments and time points sampled post-inoculation for each virus being tested. Virus titer was analyzed first by log transforming virus titer and then analyzing the data with a two-way ANCOVA using standard least squares means modeling where model effects were time post-inoculation, inoculation route, time post-inoculation*inoculation route, and queen age as a random effect. Post-hoc analyses of standard least squares means consisted of least squares means differences with Tukey HSD.

2.2.6.2.Objective 2

All statistical analyses were performed in JMP® Pro 14 (SAS Institute Inc, 2019). Queen survival was analyzed using survival analysis where time to failure is grouped by queen inoculation treatment received and analyzed by queen source, significance determined by Wilcoxon signed-rank test. Queen survival failure is defined as queen supersedure, a colony going queenless, or complete colony failure. For significant results of the survival analysis, additional

survival analyses were conducted in a pairwise manor to determine significance between the queen inoculation treatments received. Surviving queens at the end of the study were analyzed by standard least squares means models with colony and location as random effects and the following fixed effects: queen source, queen DWV-A inoculation route, tissue type, queen source*queen DWV-A inoculation route, queen source*tissue type, queen DWV-A inoculation route*tissue type, and queen source*queen DWV-A inoculation route*tissue type. Post-hoc analyses of standard least squares means consisted of least squares means differences with Tukey HSD.

Colony assessment metrics (frames of bees, frames of brood, frames of pollen, and frames of nectar) were analyzed with repeated measures three way ANCOVA using standard least squares means models with colony and location as random effects and the following fixed effects: queen source, queen DWV-A inoculation treatment, time (month sampled), and two and three way interactions between these variables. Fixed effects that were determined to be significant were further analyzed with least squares means differences Tukey HSD. Colony assessment metrics were only analyzed for queen sources Italian 202 and Italian 205; Italian 209 was excluded due to high colony mortality at the beginning of the experiment leading to little representation across all treatments. Frames of bees and frames of brood were analyzed using a univariate split-plot repeated measures approach allowing the use of earlier data points for colonies that failed before the end of the time period. Frames of pollen and nectar were analyzed using a multivariate MANOVA repeated measures due to failure to pass the test of sphericity, this data excludes colonies that did not have data for all time points.

Eggs and pupae viral analyses were split by sample type and were statistically analyzed to see if queen source and queen DWV-A inoculation treatment had any effect on the detectable levels of the viruses and the viral titer of the viruses. Detection of DWV-A, DWV-B, CBPV, and

BQCV was analyzed with logistic regression using generalized linear models with binomial distribution where the model effects were queen source, queen DWV-A inoculation route, and queen source*queen DWV-A inoculation route. Post-hoc analyses of detection consisted of categorical response analysis and response homogeneity tests with Pearson's chi-square and Fisher's exact. Virus titer was log transformed and analyzed with two-way ANCOVA using standard least squares means modeling where model effects were queen source, queen DWV-A inoculation route, queen source*queen DWV-A inoculation route, random effect of colony, and random effect of location. Post-hoc analyses of standard least squares means consisted of least squares means differences with Tukey HSD.

2.3. Results

2.3.1. Objective 1: Queen DWV-A Infection via Different Transmission Routes

The presence of DWV-A in queens was found to be significantly affected by an interaction between time after inoculation and the inoculation route, $\chi^2 = 21.684$, df = 3,3, $p = 0.0099$, where oral and venereal inoculated queens had more individuals with detectable levels of DWV-A at 1 hour compared to 7 day and 14 day non-inoculated queens, 3 day and 7 day oral inoculated queens, and 14 day oral+venereal inoculated queens (figure 2.1A). The presence of DWV-B in queens was not significantly affected by time after inoculation, $\chi^2 = 3.306$, df = 3, $p = 0.3469$, inoculation treatment received, $\chi^2 = 7.778$, df = 3, $p = 0.0508$, or the interaction between time after inoculation and the inoculation route, $\chi^2 = 6.994$, df = 3,3, $p = 0.6377$. The presence of BQCV in queens was found to be significantly affected by time after inoculation, $\chi^2 = 29.349$, df = 3, $p < 0.0001$, where 1 hour and 14 days had higher percentage of queens with detectable levels of BQCV than 3 days and 7 days (figure 2.1B). BQCV detection was not significantly affected by inoculation treatment received, $\chi^2 = 2.695 \times 10^{-8}$, df = 3, $p = 1.0000$, or the interaction between time after inoculation and

inoculation treatment received, $\chi^2 = 0.716$, df = 3,3, $p = 0.9999$. The presence of CBPV in queens was not significantly affected by time after inoculation, $\chi^2 = 0.567$, df = 3, $p = 0.9040$, inoculation treatment received, $\chi^2 = 7.356$, df = 3, $p = 0.0614$, or the interaction between time after inoculation and inoculation treatment received, $\chi^2 = 12.428$, df = 3,3, $p = 0.1902$.

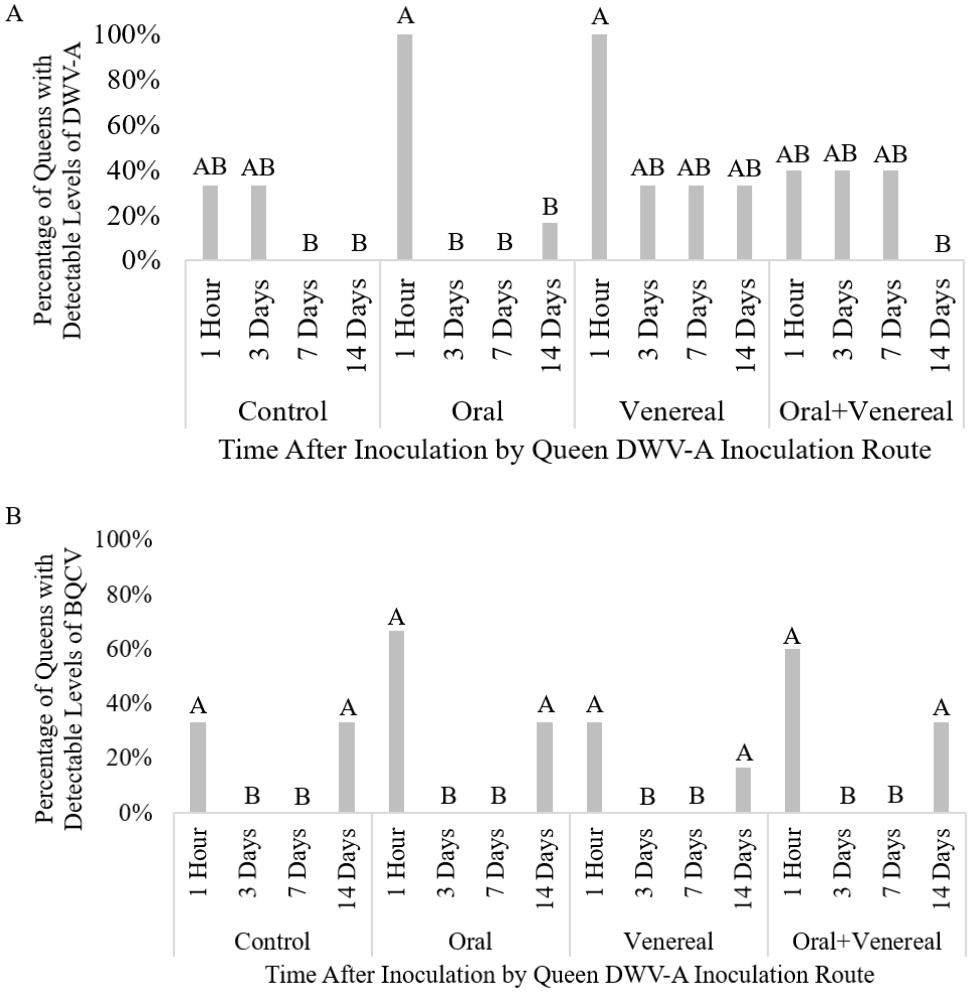

Figure 2.1. Percentage of queens with detectable levels of (A) DWV-A and (B) BQCV at four time points following inoculation with DWV-A by different transmission routes n = 93 (n = 6 for all time points and treatments, except oral+venereal 1 hour, 3 days, and 7 days, where there were only 5 samples). Bars accompanied by the same letter represent no significance ($p > 0.05$; Fisher's Exact).

DWV-A titer in queens was found to be significantly affected by an interaction between time after inoculation and the inoculation route, $F_{3,3} = 2.355$ $p = 0.0211$, where 1 hour venereal is significantly higher than 7 day and 14 day control and 3 day and 7 day oral, and all other groups

are intermediate (figure 2.2). DWV-B titer in queens was found to be significantly affected by inoculation route, $F_3 = 3.017$ $p = 0.0350$, with venereally inoculated queens ($5.875x10^9 \pm 2.297x10^9$) having higher titer than non-inoculated queens ($8.845x10^6 \pm 8.837x10^6$) and oral inoculated ($1.734x10^9 \pm 1.226x10^9$) and oral+venereal inoculated ($7.598x10^8 \pm 6.647x10^8$) being intermediate. DWV-B titer was not significantly affected by time after inoculation, $F_3 = 1.836$ $p = 0.1480$, or the interaction between time after inoculation and inoculation route, $F_{3,3} = 0.661$ $p = 0.7416$. BQCV titer in queens was found to be significantly affected by time after inoculation, $F_3 = 9.844$ $p < 0.0001$, where BQCV titer at 1 hour ($1.625x10^4 \pm 5.263x10^3$) and 14 days ($2.091x10^4 \pm 1.679x10^4$) was higher than 3 days (0.000) and 7 days (0.000). BQCV titer was not significantly affected by inoculation route, $F_3 = 0.747$ $p = 0.5274$, or the interaction between time after inoculation and inoculation route, $F_{3,3} = 0.507$ $p = 0.8651$. There was no significant effect on CBPV titer due to time after inoculation, $F_3 = 0.292$ $p = 0.8308$, inoculation treatment received, $F_3 = 2.3982$ $p = 0.0744$, or the interaction between time after inoculation and inoculation treatment received, $F_{3,3} = 1.295$ $p = 0.2535$.

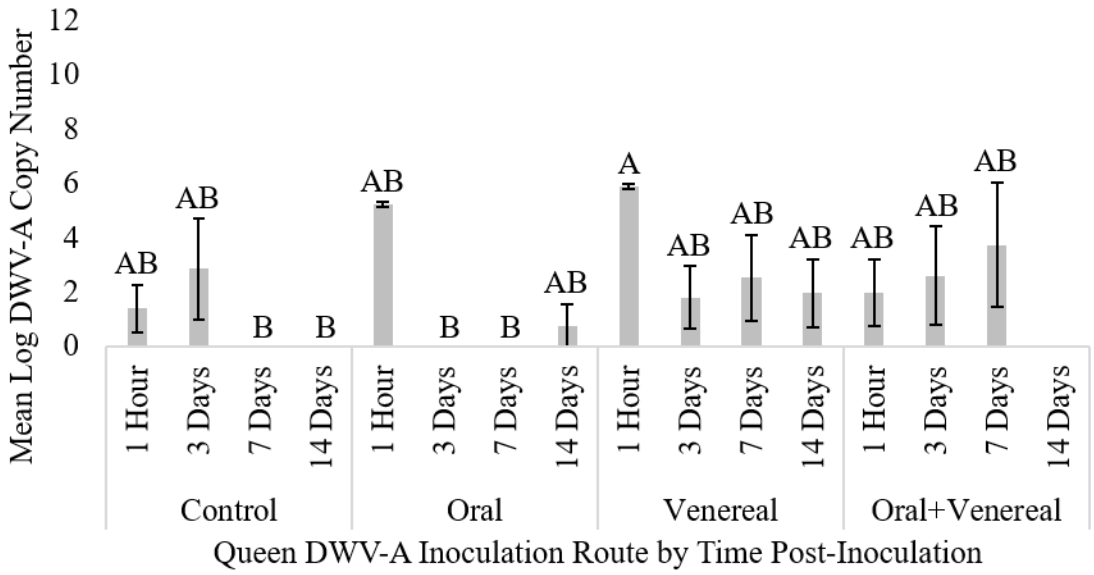

Figure 2.2. Log copy number for DWV-A at four time points following inoculation with DWV-A by different transmission routes n = 93 (n = 6 for all time points and samples, except oral+venereal 1 hour, 3 days, and 7 days, where there were only 5 samples). Bars accompanied by the same letter represent no significance ($p > 0.05$; Tukey HSD). Error bars represent one standard error from the mean.

2.3.2. Objective 2: Colony Health Following Queen Inoculation

Queen survival was not affected by queen inoculation treatment for Italian 202, $\chi^2 = 0.4915$, df = 2, $p = 0.7821$, and Italian 205, $\chi^2 = 2.8874$, df = 2, $p = 0.2361$. However there was a significant effect of queen inoculation treatment on queen survival in queen source Italian 209, $\chi^2 = 10.6682$, df = 2, $p = 0.0048$ (figure 2.3). Within queen source Italian 209, there was no significance between oral and venereal queen inoculation, $\chi^2 = 1.0204$, df = 1, $p = 0.3124$, but there was a significant difference between non-inoculated queens and venereally inoculated queens, $\chi^2 = 5.8053$, df = 1, $p = 0.0160$, and orally inoculated queens, $\chi^2 = 9.7550$, df = 1, $p = 0.0018$ (figure 2.3C).

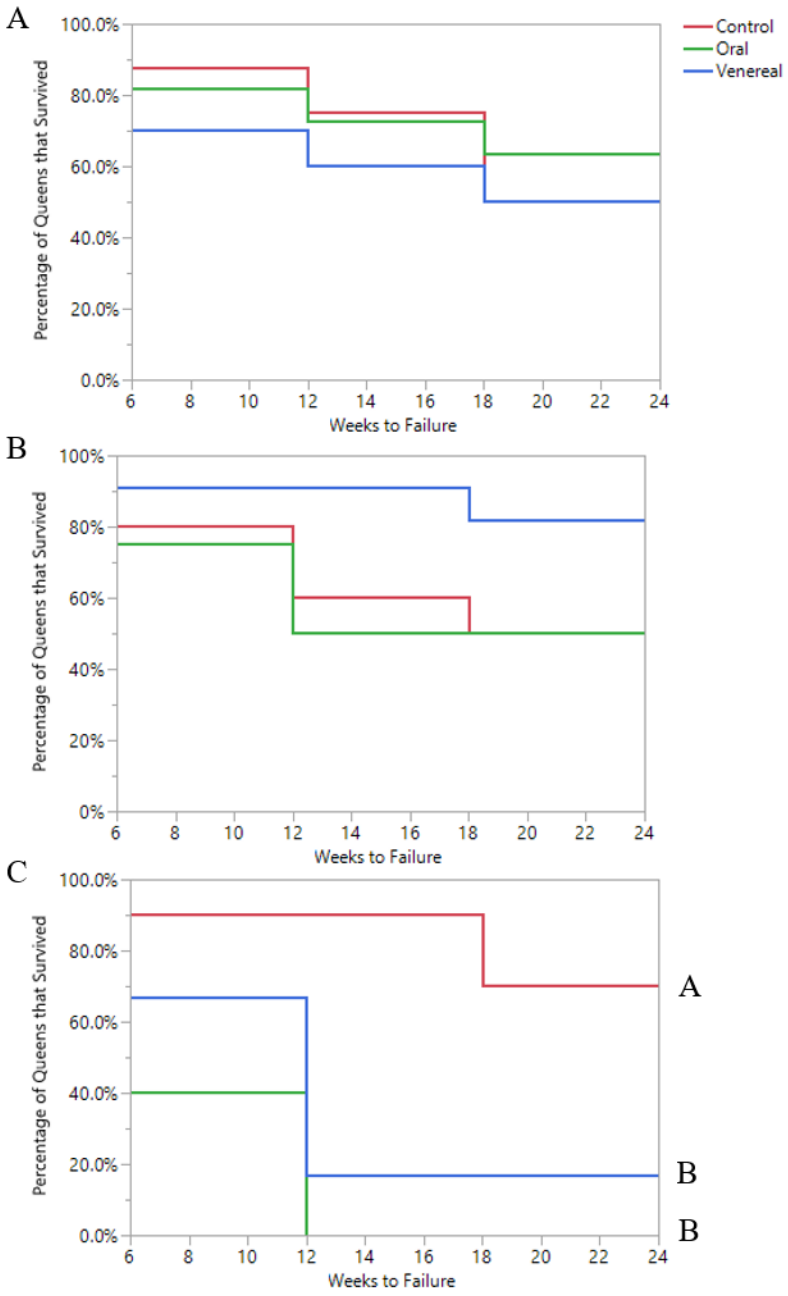

Figure 2.3. Queen survival over 24 weeks after DWV-A inoculation and colony introduction for different DWV-A inoculation routes within different queen sources (A) Italian 202 n = 29 (n_C = 8, n_O = 11, n_V = 10), (B) Italian 205 n = 29 (n_C = 10, n_O = 8, n_V = 11), and (C) Italian 209 n = 21 (n_C = 10, n_O = 5, n_V = 6). Lines accompanied by the same letter represent no significance ($p > 0.05$).

Queens that survived to the end of the field season had no detectable levels of DWV-A. However, queens did have detectable levels of BQCV. There was a significant difference in BQCV titer between the tissue types analyzed, $F_3 = 58.426$ $p < 0.0001$, with the gut ($1.147 \times 10^6 \pm$

$7.217x10^5$) having a higher titer than the body ($3.656x10^3 \pm 1.651x10^3$), and the body having a higher titer than the ovaries (0.000) and spermatheca (0.000). BQCV titer was not significantly affected by queen source, $F_1 = 0.042$ $p = 0.8416$, queen DWV-A inoculation route, $F_2 = 0.516$ $p = 0.6077$, or any two or three-way interactions ($p > 0.1$ for all comparisons; figure A3).

There was a random effect due to colony on frames of bees, $p = 0.0003$, but no random effect due to location, $p = 0.5995$. Frames of bees was significantly affected by queen DWV-A inoculation route, $F_2 = 3.246$ $p = 0.0491$, where colonies with orally inoculated queens (4.34 ± 0.29) had a higher average frames of bees than colonies with non-inoculated queens (3.31 ± 0.21) or venereally inoculated queens (3.31 ± 0.19) (figure 2.4). Frames of bees was also significantly affected by the month they were sampled, $F_2 = 26.982$ $p < 0.0001$, with July (4.48 ± 0.31) being significantly higher than June (3.17 ± 0.18) and September (3.29 ± 0.23) (figure 2.4). The following did not have a significant effect on frames of bees: queen source, $F_1 = 0.142$ $p = 0.7082$, or two or three-way interactions ($p > 0.1$ for all comparisons; table A.4).

There was a random effect due to colony on frames of brood, $p = 0.0081$, but no random effect due to location, $p = 0.9030$. Frames of brood was significantly affected by an interaction between time and queen DWV-A inoculation route, $F_{2,2} = 3.376$ $p = 0.0144$, where June had higher averages of frames of bees when comparing the same route of inoculation across the three time points except for colonies with venereal inoculated queens, which did not significantly differ in average frames of bees over the three month period (figure 2.5). Frames of brood were also affected by an interaction between queen source and queen DWV-A inoculation route, $F_{1,2} = 3.943$ $p = 0.0286$, however multiple comparisons with least square means differences Tukey HSD showed no significant differences for multiple comparisons. Frames of brood were not significantly affected by any other two or three-way interactions ($p > 0.1$ for all comparisons; table A.4).

There were no random effects due to colony, $p = 0.1775$, or location, $p = 0.5224$. Frames of pollen were significantly affected by time, $F_2 = 6.344$ $p = 0.0053$, with September (0.19 ± 0.03) being significantly higher than June (0.07 ± 0.01) and July (0.09 ± 0.02) (figure 2.6). Frames of pollen was not significantly affected by queen source, $F_1 = 0.317$ $p = 0.5780$, queen DWV-A inoculation route, $F_2 = 1.717$ $p = 0.1974$, or any two or three-way interactions ($p > 0.1$ for all comparisons; table A.4).

There was a random effect due to colony on frames of nectar, $p = 0.0002$, but no random effect due to location, $p = 0.8598$. Frames of nectar were significantly affected by an interaction between time and queen source, $F_{2,1} = 4.560$ $p = 0.0193$, where July and September had higher frames of nectar that June for both queen sources, and within queen source Italian 205 July and September significantly differed but within Italian 202 they did not (figure 2.7). Frames of nectar was not significantly affected by queen DWV-A inoculation route, $F_2 = 1.722$ $p = 0.1964$, or any other two or three way interactions ($p > 0.1$ for all comparisons; table A.4).

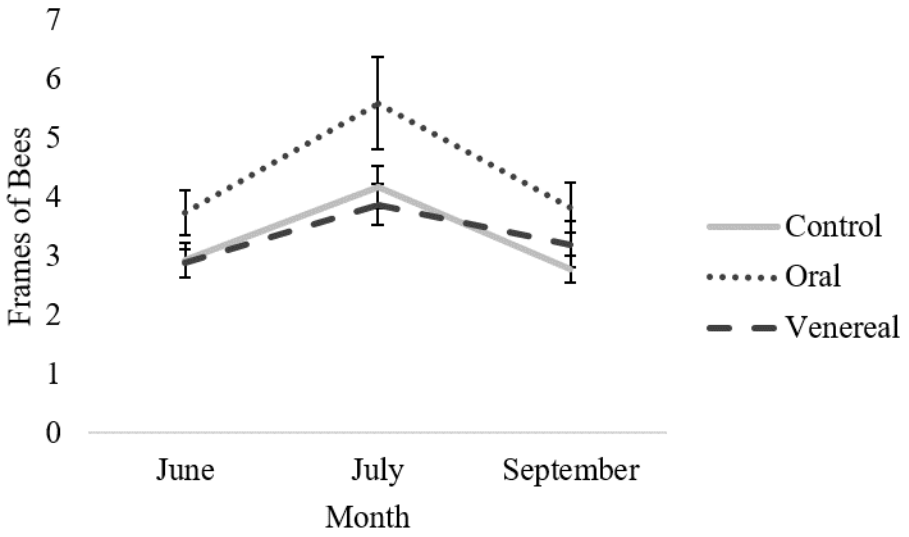

Figure 2.4. Frames of bees over time for colonies headed by queens inoculated with DWV-A through different transmission routes n = 47 colonies ($n_{ControlJune} = 15$, $n_{ControlJuly} = 12$, $n_{ControlSept} = 9$, $n_{OralJune} = 15$, $n_{OralJuly} = 12$, $n_{OralSept} = 11$, $n_{VenerealJune} = 17$, $n_{VenerealJuly} = 16$, $n_{VenerealSept} = 15$). Error bars represent one standard error from the mean.

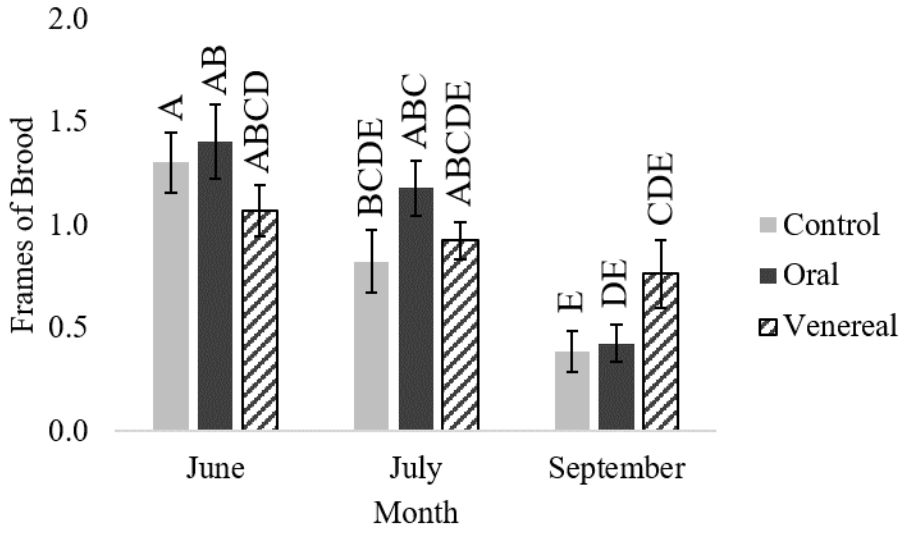

Figure 2.5. Frames of brood over time for colonies headed by queens inoculated with DWV-A through different transmission routes n = 47 colonies ($n_{ControlJune}$ = 15, $n_{ControlJuly}$ = 12, $n_{ControlSept}$ = 9, $n_{OralJune}$ = 15, $n_{OralJuly}$ = 12, $n_{OralSept}$ = 11, $n_{VenerealJune}$ = 17, $n_{VenerealJuly}$ = 16, $n_{VenerealSept}$ = 15). Bars accompanied by the same letter represent no significance ($p > 0.05$; Tukey HSD). Error bars represent one standard error from the mean.

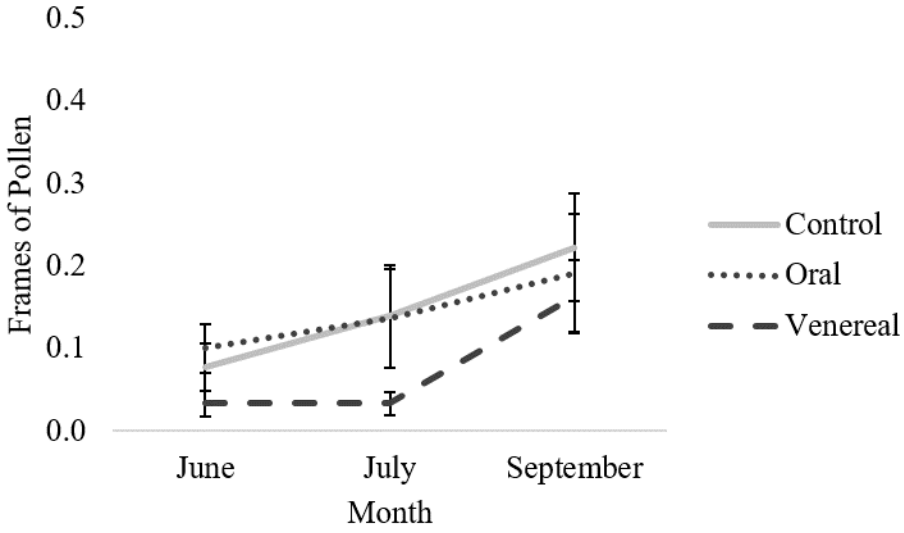

Figure 2.6. Frames of pollen over time for colonies headed by queens inoculated with DWV-A through different transmission routes n = 35 colonies ($n_{Control}$ = 9, n_{Oral} = 11, $n_{Venereal}$ = 15). Error bars represent one standard error from the mean.

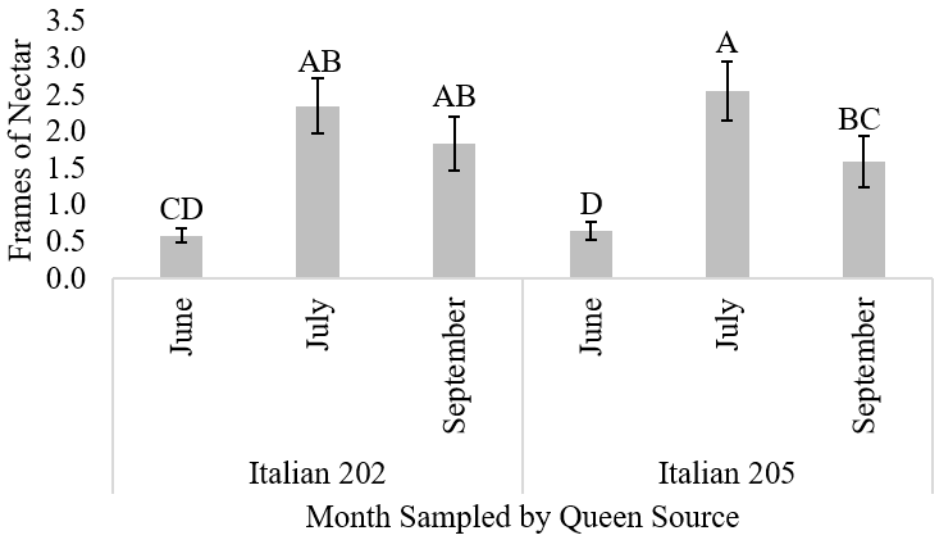

Figure 2.7. Frames of nectar over time for colonies headed by queens inoculated with DWV-A through different transmission routes n_{202} = 18 colonies per bar and n_{205} = 17 colonies per bar. Bars accompanied by the same letter represent no significance ($p > 0.05$; Tukey HSD). Error bars represent one standard error from the mean.

Eggs sampled and tested for honey bee viruses had varying virus presence and titer between the different viruses tested. Only 1 out of 45 eggs tested had detectable levels of DWV-A with a DWV-A titer of 3.4241×10^{4}. Due to the low number of samples with detectable levels of the virus no statistical analyses were run on these samples. For DWV-B, 44 out of 56 eggs tested had detectable levels of DWV-B. The presence of DWV-B was not significantly affected by queen source, $\chi^2 = 5.818 \times 10^{-7}$, df = 1, $p = 0.9994$, queen DWV-A inoculation route, $\chi^2 = 3.586 \times 10^{-6}$, df = 2, $p = 1.0000$, or the interaction between queen source and queen DWV-A inoculation route, $\chi^2 = 4.035 \times 10^{-6}$, df = 2, $p = 1.0000$ (figure 2.6A). DWV-B titer was not significantly affected by random effects due to colony, $p = 0.6906$, or location, $p = 0.8049$. DWV-B titer was not significantly affected by queen source, $F_1 = 1.617$ $p = 0.2111$, queen DWV-A inoculation route, $F_2 = 1.216$ $p = 0.3075$, or the interaction between queen source and queen DWV-A inoculation route, $F_{1,2} = 0.381$ $p = 0.6860$ (figure 2.6A). For CBPV, 0 out of 45 eggs had detectable levels of CBPV, and therefore no titer values either. Due to the low number of samples with detectable levels of the virus no

statistical analyses were run on these samples. For BQCV, 14 out of 45 eggs had detectable levels of BQCV. The presence of BQCV was significantly affected by queen DWV-A inoculation route, χ^2 = 17.633, df = 2, p = 0.0001, where queens inoculated with DWV-A venereally were significantly different from non-inoculated queens, χ^2 = 15.010, df = 1, p = 0.0001, and orally inoculated queens, χ^2 = 4.747, df = 1, p = 0.0293 (figure 2.6B). BQCV presence was not significantly affected by queen source, χ^2 = 2.388, df = 1, p = 0.1223, or queen source*queen DWV-A inoculation route, χ^2 = 5.344, df = 1,2, p = 0.0691. There was no significant random effects on BQCV titer due to colony, p = 0.3546, or location, p = 0.8552. BQCV titer was significantly affected by queen DWV-A inoculation route, F_2 = 9.498 p = 0.0004, where queens venereally inoculated with DWV-A were significantly different from non-inoculated queens and orally inoculated queens (figure 2.6B). There was no significant difference in BQCV titer due to queen source, F_1 = 0.324 p = 0.5722, or queen source*queen DWV-A inoculation route, $F_{1,2}$ = 1.513 p = 0.2329.

Pupae sampled and tested for honey bee viruses had varying virus presence and titer between the different viruses tested. None of the pupae tested had detectable levels of DWV-A, and therefore no titer values either. For DWV-B, 3 out of 42 pupae had detectable levels of DWV-B. For CBPV, 1 out of 42 pupae had detectable levels of CBPV. For BQCV, 0 out of 42 pupae had detectable levels of BQCV and therefore no titer values either. Due to the low number of samples with detectable levels of any of the viruses tested for no statistical analyses were run on these samples.

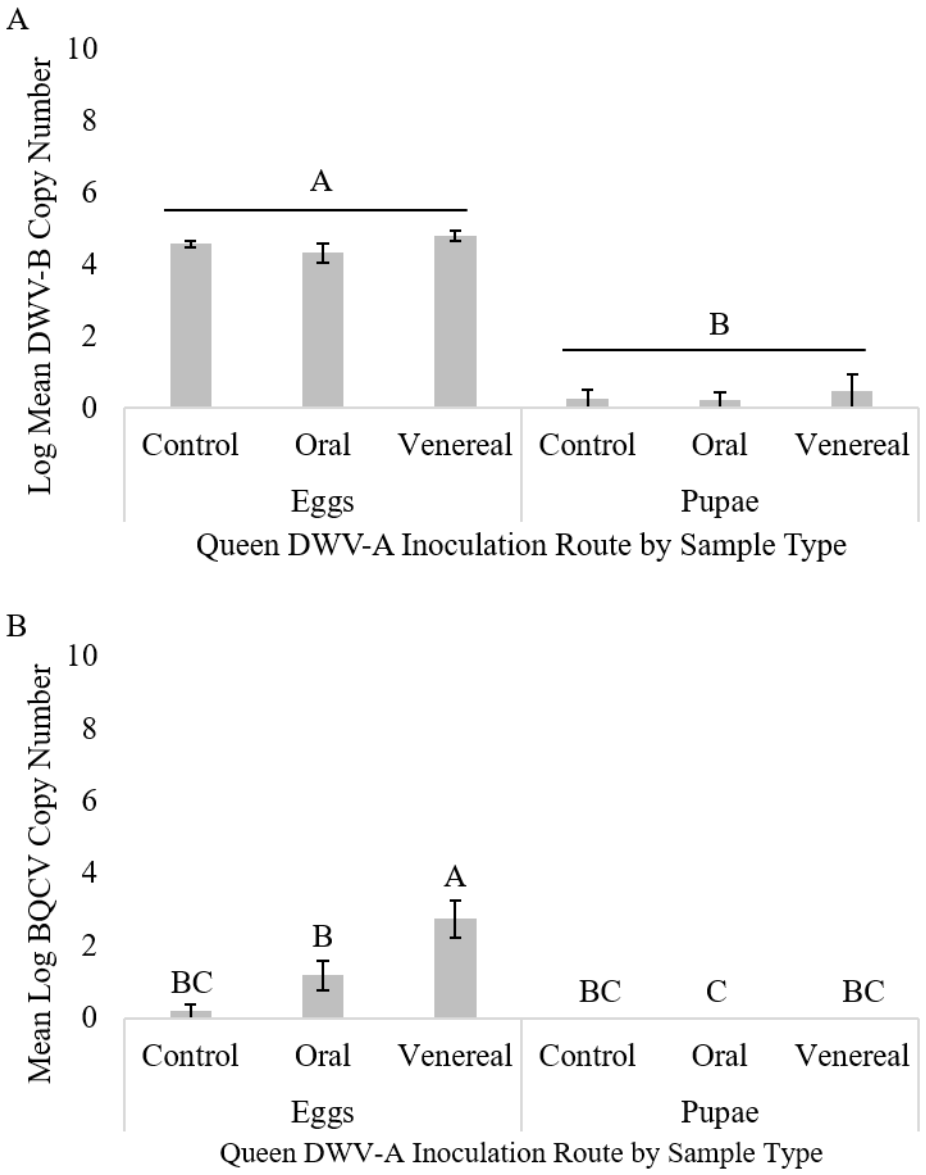

Figure 2.8. Log copy number of (A) DWV-B and (B) BQCV for two brood stages originating from honey bee queens inoculated with DWV-A through different transmission routes n = 87 (n_{EggC} = 18, n_{EggO} = 18, n_{EggV} = 9, n_{PupaC} = 15, n_{PupaO} = 18, n_{PupaV} = 9). Bars accompanied by the same letter represent no significance ($p > 0.05$; Tukey HSD). Error bars represent one standard error from the mean.

2.4. Discussion

Our goal was to determine how the transmission route of DWV-A in honey bee queens affected virus infection and progression, as well as how the different transmission routes could impact the strength and health of colonies headed by virus exposed queens. Our results suggest

that honey bee queens may be able to prevent virus infection and that colony survival can be impacted by the route of DWV-A inoculation in queens based on differing genetic backgrounds. We showed that artificial inoculation with DWV-A through oral and venereal transmission was successful in delivering the virus to the queens, but none of the routes tested consistently led to established high virus titer infections. Colony survival was significantly lower in one queen source for queens inoculated with DWV-A compared to two different queen sources that showed no significant difference in colony survival due to inoculation with DWV-A. Overall colony strength and health was unaffected by the queen's DWV-A inoculation through oral or venereal transmission routes. These findings suggest that there is no difference between the transmission routes of DWV-A on queen and colony health , but that the transmission of the virus to honey bee queens can negatively impact colony survival based on some queen genotypes implying that there is a genetic component involved in the host-pathogen relationship that needs to be considered.

We found that venereal transmission of DWV-A led to a more consistent virus presence at all time points post-inoculation in honey bee queens compared to oral transmission, suggesting that venereal transmission may lead to infection more readily than oral transmission. We hypothesize that this difference between transmission routes may be due to the different barriers the virus must overcome to establish within its host. Transmission of the virus through contaminated semen may have a greater potential for the virus to establish within honey bee queens because the virus is being directly delivered to the reproductive organs, whereas with oral transmission the virus needs to circumvent physical barriers within the insect gut (Amiri et al., 2016). However, data and previous research has shown that venereal transmission of DWV does not always lead to high titer virus infection (Amiri et al., 2016). Honey bee queens may be capable of preventing virus infection due to venereal transmission potentially by purging seminal fluid

which occurs after mating due to excess amount of semen and a limited spermatheca capacity, potentially removing virus contaminated semen in the process (Ruttner, 1956; Winston, 1991). This process is likely insufficient in fully eliminating virus exposure through venereal transmission due to the necessity for queens to maintain large quantities of viable sperm for brood production throughout her lifespan. Drosophila have demonstrated upregulation of a local antimicrobial peptide synthesis in the reproductive tract in response to mating which may aid in preventing infections, it is possible that honey bees may have a similar mechanism but this has yet to be studied (Ferrandon et al., 1998; Peng et al., 2005; Tzou et al., 2000). Therefore, queens appear to be capable of some physiological defense mechanism against virus infection from venereal transmission, further research is needed to determine what is responsible for this.

Oral transmission of viruses may be less likely to establish into an infection due to multiple barriers within the insect gut that defend against pathogens. The insect gut features physical, cellular, and humoral defenses against viruses via the peritrophic membrane, cellular apoptosis, and antiviral immune response (Keehnen et al., 2017; Liu et al., 2017). Due to the multiple layers of defense, it is more difficult for viruses to infect insects through oral transmission, however some pathogens have evolved mechanisms of bypassing some of these defenses (Liu et al., 2017). Our data shows that single exposure oral transmission of DWV-A was not able to establish within inoculated queens, which may be due to the innate insect defense against oral transmission of viruses or an unknown mechanism in honey bees that has yet to be determined, multiple exposures of the virus through oral transmission needs to be studied.

Three additional viruses were examined during the queen inoculation experiment and we found that DWV-B and CBPV showed no difference in detection and titer level at any time point for any of the DWV-A inoculation treatments, but BQCV showed that time post-inoculation with

DWV-A significantly influenced the detection of the virus. DWV-B and CBPV were detected at low titer levels for all DWV-A inoculation routes and for all time points after inoculation with DWV-A, indicating a low-level background presence across all queen treatments. BQCV showed a reduction in virus detection and titer at 3 days and 7 days regardless of exposure to DWV-A, suggesting that these queens were able to resist infection with BQCV through immune response mechanisms not utilized in immune response that targets DWV-A infections (Brutscher et al., 2015). BQCV reappeared at 14 days either due to repeated exposure to the virus within the colony or an artifact of subsampling. This data suggests that inoculation with DWV-A does not influence the presence or progression of these other viruses.

Queen sources differed in susceptibility to DWV-A exposure, leading to increased colony loss for one queen source and no significant impact for two other queen sources. The route of transmission did not differ in colony survival, the significance in colony survival was between non-inoculated queens and inoculated queens. Queens were reared from three different source colonies which differed in genetic background, as well as food resources and exposure to pathogens during the 1st instar period prior to being grafted and placed into the same colony setting. Whether one of these factors or all three are responsible for the difference in susceptibility is unknown, but previous research has shown differences in susceptibility to viruses for workers from different colonies and different genetic stocks (Boncristiani et al., 2013; Khongphinitbunjong et al., 2015; Kulinčević & Rothenbuhler, 1975; Rinderer et al., 1975). In addition to these differences, there could also have been a higher susceptibility in one queen source due to suppressed immune response or synergistic effects between multiple pathogens and other stressors (Nazzi et al., 2012; Yang & Cox-Foster, 2005; Zheng et al., 2015). Potential evidence that this may have played a role for Italian 209 queens was found when molecular analyses were conducted

on a non-inoculated Italian 209 queen not used in the study, finding DWV-A at 10^9, DWV-B at a high titer, and CBPV at a low titer (data not shown). The presence of these high virus titers, possibly from vertical transmission or oral transmission as 1st instar larvae prior to grafting, in addition to experimental inoculation could have led to the high colony losses observed for Italian 209. This is further supported through viral analysis of surviving queens at the end of the field season having no detection for DWV-A, suggesting these queens, who were from source Italian 202 and Italian 205, may have been better at preventing virus infection than source Italian 209 or queens from Italian 202 and Italian 205 did not start with high virus levels from vertical transmission which is likely what led to the high colony losses experienced for Italian 209. Therefore, breeding of queens should be carefully monitored by avoiding weaker/sickly colonies which may be more susceptible to pathogen exposure than other queen sources.

Inoculating queens with DWV-A did not lead to DWV-A infected eggs or pupae, however DWV-B and BQCV were found in eggs but not pupae. We also observed significantly more eggs with detectable levels of BQCV and at higher titer levels from queens venereally inoculated with DWV-A than non-inoculated and orally inoculated queens. The semen used to inseminate queens contained minimal levels of CBPV and DWV-B, and moderate levels of BQCV. This explains the detection of DWV-B and BQCV in the eggs analyzed. BQCV detection was higher in eggs laid by queens venereally inoculated with DWV-A, showing that venereal exposure to multiple viruses may lead to increased virus presence in eggs laid by queens. These findings imply that venereal transmission of viruses could play a significant role in vertical transmission of viruses when semen is contaminated by multiple pathogens. However, our data shows that vertical transmission of viruses may not lead to infection in developing brood. Eggs analyzed showed DWV-B and BQCV was detected, however pupae sampled from the same colonies showed little to no virus detection.

These findings support previous research suggesting vertical transmission of viruses may not lead to substantial disease spread and brood infection since the virus adheres to the surface of the egg, rather than within the egg (Amiri et al., 2018). Another possibility is immune response preventing low level virus exposure from becoming a true infection. Based on our findings and previous research, we propose that honey bee queens are potentially a less significant source of disease spread within a colony via vertical transmission of viruses. In addition to the egg and pupae virus data, our data also shows that queen exposure to DWV-A may not significantly affect colony strength metrics and that changes in these metrics are likely due to temporal patterns of colony metric fluctuations (Winston, 1991). Based on these findings, we conclude that a single exposure of DWV-A to honey bee queens does not negatively impact colony strength or colony health unless the queen is of a susceptible genotype which results in colony failure, however further work is needed to assess the consequences of repeated DWV-A exposure.

The findings of this study may have been influenced by limitations such as limited sample sizes for queen inoculation and limited number of colonies for colony strength and health, low RNA yields for a portion of queens during the queen inoculation experiment, and timing and location limitations for the field portion of the study. RNA yields from extraction were lower for 30% of the 7 day queens across all treatment groups and all of the 14 day queens, which may have impacted PCR results due to unequal cDNA concentrations after cDNA synthesis. According to the Qiagen QuantiTect Reverse Transcription Kit product page, the differences in cDNA yield would result in the samples with lower RNA input having on average a higher C_T value of about 3.5 (Qiagen, 2019). This difference in C_T value translates to a one fold titer level difference for DWV-A, DWV-B, and BQCV. This reduces the ability to directly compare 14 day queens to the other time points, but the range of variation in samples indicates this wouldn't have strongly

impacted results, therefore further research is needed to increase sample size and reduce variation. The field study was initiated in late spring leading to small, underdeveloped colonies with low food resources. These negative effects led to vulnerability to pests, further weakening colonies. In addition, the study had to be split into two different apiaries 7.21km apart due to the size of the study, which resulted in different forage availability and diversity since there was not overlap in forage range and foraging mostly occurs within 6km of colonies (Winston, 1991).

In summary, transmission route of DWV-A does not affect queen or colony health for single exposure, but that exposure to DWV-A may lead to colony loss in some queen sources that are more susceptible. Our results suggest that honey bee queens are able to prevent virus infection when exposed to DWV-A orally and/or venereally, and that colony strength metrics and brood health are not impacted by exposure to DWV-A regardless of transmission route. In addition, our results indicate that some queens may be more susceptible to DWV-A exposure due to potentially genetics and previous exposure to pathogens during rearing, leading to increased levels of colony loss. These conclusions suggest that although DWV-A transmission to queens is not inherently problematic, but could lead to colony loss due to differences in queen sources. These findings encourage beekeepers to rear queens from multiple sources or from known strong queens and colonies to avoid colony loss.

2.5. References

Amiri, E., Kryger, P., Meixner, M. D., Strand, M. K., Tarpy, D. R., & Rueppell, O. (2018). Quantitative patterns of vertical transmission of deformed wing virus in honey bees. *PLOS One, 13*(3), e0195283.

Amiri, E., Meixner, M. D., & Kryger, P. (2016). Deformed wing virus can be transmitted during natural mating in honey bees and infect the queens. *Scientific Reports, 6*.

Amiri, E., Strand, M. K., Rueppell, O., & Tarpy, D. R. (2017). Queen Quality and the Impact of Honey Bee Diseases on Queen Health: Potential for Interactions between Two Major Threats to Colony Health. *Insects, 8*(2), 48.

Blanchard, P., Ribière, M., Celle, O., Lallemand, P., Schurr, F., Olivier, V., . . . Faucon, J. P. (2007). Evaluation of a real-time two-step RT-PCR assay for quantitation of Chronic bee paralysis virus (CBPV) genome in experimentally-infected bee tissues and in life stages of a symptomatic colony. *Journal of Virological Methods, 141*(1), 7-13.

Boncristiani, H. F., Evans, J. D., Chen, Y., Pettis, J., Murphy, C., Lopez, D. L., . . . Rueppell, O. (2013). In vitro infection of pupae with Israeli acute paralysis virus suggests disturbance of transcriptional homeostasis in honey bees (Apis mellifera). *PLOS One, 8*(9), e73429.

Brutscher, L. M., Daughenbaugh, K. F., & Flenniken, M. L. (2015). Antiviral defense mechanisms in honey bees. *Current Opinion in Insect Science, 10*, 71-82.

Büchler, R., Andonov, S., Bienefeld, K., Costa, C., Hatjina, F., Kezic, N., . . . Wilde, J. (2013). Standard methods for rearing and selection of Apis mellifera queens. *Journal of Apicultural Research, 52*(1), 1-30.

Chen, Y., Evans, J., & Feldlaufer, M. (2006). Horizontal and vertical transmission of viruses in the honey bee, Apis mellifera. *Journal of Invertebrate Pathology, 92*(3), 152-159.

Chen, Y., Pettis, J. S., Collins, A., & Feldlaufer, M. F. (2006). Prevalence and transmission of honeybee viruses. *Applied and Environmental Microbiology, 72*(1), 606-611.

Chen, Y., Pettis, J. S., & Feldlaufer, M. F. (2005). Detection of multiple viruses in queens of the honey bee Apis mellifera L. *Journal of Invertebrate Pathology, 90*(2), 118-121.

Cobey, S. W., Tarpy, D. R., & Woyke, J. (2013). Standard methods for instrumental insemination of Apis mellifera queens. *Journal of Apicultural Research, 52*(4), 1-18.

Daughenbaugh, K., Martin, M., Brutscher, L., Cavigli, I., Garcia, E., Lavin, M., & Flenniken, M. (2015). Honey bee infecting Lake Sinai viruses. *Viruses, 7*(6), 3285-3309.

de Guzman, L. I., Frake, A. M., & Simone-Finstrom, M. (2017). Comparative Flight Activities and Pathogen Load of Two Stocks of Honey Bees Reared in Gamma-Irradiated Combs. *Insects, 8*(4), 127.

de Guzman, L. I., Simone-Finstrom, M., Frake, A. M., & Tokarz, P. (2019). Comb irradiation has limited, interactive effects on colony performance or pathogens in bees, Varroa destructor and wax based on two honey bee stocks. *Insects, 10*(1), 15.

Delaplane, K. S., van der Steen, J., & Guzman-Novoa, E. (2013). Standard methods for estimating strength parameters of Apis mellifera colonies. *Journal of Apicultural Research, 52*(1), 1-12.

De Miranda, J. R., Bailey, L., Ball, B. V., Blanchard, P., Budge, G. E., Chejanovsky, N., . . . De Graaf, D. C. (2013). Standard methods for virus research in Apis mellifera. *Journal of Apicultural Research, 52*(4), 1-56.

De Miranda, J., & Fries, I. (2008). Venereal and vertical transmission of deformed wing virus in honeybees (Apis mellifera L.). *Journal of Invertebrate Pathology, 98*(2), 184-189.

Dietemann, V., Nazzi, F., Martin, S. J., Anderson, D. L., Locke, B., Delaplane, K. S., . . . Ziegelmann, B. (2013). Standard methods for varroa research. *Journal of Apicultural Research, 52*(1), 1-54.

Ellis, J. D., Evans, J. D., & Pettis, J. (2010). Colony losses, managed colony population decline, and Colony Collapse Disorder in the United States. *Journal of Apicultural Research, 49*(1), 134-136.

Ferrandon, D., Jung, A., Criqui, M. C., Lemaitre, B., Uttenweiler-Joseph, S., Michaut, L., . . . Hoffmann, J. (1998). A drosomycin–GFP reporter transgene reveals a local immune response in Drosophila that is not dependent on the Toll pathway. *The EMBO Journal, 17*(5), 1217-1227.

Francis, R., & Kryger, P. (2012). Single assay detection of acute bee paralysis virus, Kashmir bee virus and Israeli acute paralysis virus. *Journal of Apicultural Science, 56*(1), 137-146.

Francis, R. M., Nielsen, S. L., & Kryger, P. (2013). Patterns of viral infection in honey bee queens. *Journal of General Virology, 94*(3), 668-676.

Gauthier, L., Ravallec, M., Tournaire, M., Cousserans, F., Bergoin, M., Dainat, B., & De Miranda, J. R. (2011). Viruses associated with ovarian degeneration in Apis mellifera L. queens. *PLOS One, 6*(1), e16217.

Gisder, S., Aumeier, P., & Genersch, E. (2009). Deformed wing virus: replication and viral load in mites (Varroa destructor). *Journal of General Virology, 90*(2), 463-467.

Hou, Y., Zhang, H., Miranda, L., & Lin, S. (2010). Serious overestimation in quantitative PCR by circular (supercoiled) plasmid standard: microalgal pcna as the model gene. *PLOS One, 5*(3), e9545.

Keehnen, N. L., Rolff, J., Theopold, U., & Wheat, C. W. (2017). Insect antimicrobial defences: A brief history, recent findings, biases, and a way forward in evolutionary studies *Advances in Insect Physiology* (Vol. 52, pp. 1-33): Elsevier.

Kevill, J. L., de Souza, F. S., Sharples, C., Oliver, R., Schroeder, D. C., & Martin, S. J. (2019). DWV-A lethal to honey bees (Apis mellifera): a colony level survey of DWV variants (A, B, and C) in England, Wales, and 32 states across the US. *Viruses, 11*(5), 426.

Khongphinitbunjong, K., de Guzman, L. I., Tarver, M. R., Rinderer, T. E., Chen, Y., & Chantawannakul, P. (2015). Differential viral levels and immune gene expression in three stocks of Apis mellifera induced by different numbers of Varroa destructor. *Journal of Insect Physiology, 72*, 28-34.

Kulinčević, J. M., & Rothenbuhler, W. C. (1975). Selection for resistance and susceptibility to hairless-black syndrome in the honeybee. *Journal of Invertebrate Pathology, 25*(3), 289-295.

Lanzi, G., De Miranda, J. R., Boniotti, M. B., Cameron, C. E., Lavazza, A., Capucci, L., . . . Rossi, C. (2006). Molecular and biological characterization of deformed wing virus of honeybees (Apis mellifera L.). *Journal of Virology, 80*(10), 4998-5009.

Liu, F., Huang, W., Wu, K., Qiu, Z., Huang, Y., & Ling, E. (2017). Exploiting innate immunity for biological pest control *Advances in Insect Physiology* (Vol. 52, pp. 199-230): Elsevier.

Möckel, N., Gisder, S., & Genersch, E. (2011). Horizontal transmission of deformed wing virus: pathological consequences in adult bees (Apis mellifera) depend on the transmission route. *Journal of General Virology, 92*(2), 370-377.

Nazzi, F., Brown, S. P., Annoscia, D., Del Piccolo, F., Di Prisco, G., Varricchio, P., . . . Pennacchio, F. (2012). Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. *PLoS Pathogens, 8*(6), e1002735.

Peng, J., Zipperlen, P., & Kubli, E. (2005). Drosophila sex-peptide stimulates female innate immune system after mating via the Toll and Imd pathways. *Current Biology, 15*(18), 1690-1694.

Promega. (2017). *Maxwell 16 LEV simplyRNA Tissue Kit: Technical Manual*

Qiagen. (2019). QuantiTect Reverse Transcription Kit Product Details.

Rinderer, T. E., Rothenbuhler, W. C., & Kulinčević, J. M. (1975). Responses of three genetically different stocks of the honeybee to a virus from bees with hairless-black syndrome. *Journal of Invertebrate Pathology, 25*(3), 297-300.

Rinkevich, F. D., Danka, R. G., & Healy, K. B. (2017). Influence of varroa mite (Varroa destructor) management practices on insecticide sensitivity in the honey bee (Apis mellifera). *Insects, 8*(1), 9.

Ruttner, F. (1956). The mating of the honeybee. *Bee World, 37*(1), 3-15.

Ryabov, E. V., Childers, A. K., Chen, Y., Madella, S., Nessa, A., & Evans, J. D. (2017). Recent spread of Varroa destructor virus-1, a honey bee pathogen, in the United States. *Scientific Reports, 7*(1), 17447.

SAS Institute Inc. (2019). JMP® Pro (Version 14). Cary, NC.

Seitz, N., Traynor, K. S., Steinhauer, N., Rennich, K., Wilson, M. E., Ellis, J. D., . . . Caron, D. M. (2015). A national survey of managed honey bee 2014–2015 annual colony losses in the USA. *Journal of Apicultural Research, 54*(4), 292-304.

Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M. E., & Bergoin, M. (2004). Prevalence and seasonal variations of six bee viruses in Apis mellifera L. and Varroa destructor mite populations in France. *Applied and Environmental Microbiology, 70*(12), 7185-7191.

Traynor, K. S., Rennich, K., Forsgren, E., Rose, R., Pettis, J., Kunkel, G., . . . Lopez, D. (2016). Multiyear survey targeting disease incidence in US honey bees. *Apidologie, 47*(3), 325-347.

Tzou, P., Ohresser, S., Ferrandon, D., Capovilla, M., Reichhart, J.-M., Lemaitre, B., . . . Imler, J.-L. (2000). Tissue-specific inducible expression of antimicrobial peptide genes in Drosophila surface epithelia. *Immunity, 13*(5), 737-748.

Villa, J. D., & Danka, R. G. (2005). Caste, sex and strain of honey bees (Apis mellifera) affect infestation with tracheal mites (Acarapis woodi). *Experimental & Applied Acarology, 37*(3-4), 157.

Webster, T. C., Pomper, K. W., Hunt, G., Thacker, E. M., & Jones, S. C. (2004). Nosema apis infection in worker and queen Apis mellifera. *Apidologie, 35*(1), 49-54.

Wilfert, L., Long, G., Leggett, H., Schmid-Hempel, P., Butlin, R., Martin, S., & Boots, M. (2016). Deformed wing virus is a recent global epidemic in honeybees driven by Varroa mites. *Science, 351*(6273), 594-597.

Winston, M. L. (1991). *The Biology of the Honey Bee*: Harvard University Press.

Yang, X., & Cox-Foster, D. L. (2005). Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host immunosuppression and viral amplification. *Proceedings of the National Academy of Sciences of the United States of America, 102*(21), 7470-7475.

Yoo, M., Kim, I., Kang, M., Han, S., & Yoon, B. (2008). Development of real-time PCR method for black queen cell virus. *Korean Journal of Apiculture*.

Yue, C., & Genersch, E. (2005). RT-PCR analysis of Deformed wing virus in honeybees (Apis mellifera) and mites (Varroa destructor). *Journal of General Virology, 86*(12), 3419-3424.

Zheng, H.-Q., Gong, H.-R., Huang, S.-K., Sohr, A., Hu, F.-L., & Chen, Y. P. (2015). Evidence of the synergistic interaction of honey bee pathogens Nosema ceranae and deformed wing virus. *Veterinary Microbiology, 177*(1-2), 1-6.

CHAPTER 3. TRANSGENERATIONAL IMMUNE PRIMING

3.1. Introduction

The social behavior and large colony sizes of honey bees (*Apis mellifera*) provides an ideal environment to study pathogen survival and defense. Despite being a prime environment for disease prevalence, honey bee colonies are able to maintain high populations of functional bees (Naug & Camazine, 2002). Although social insects have various social and behavioral tactics for fighting off disease, there are also immune functions that contribute to their ability to withstand a pathogen outbreak. The honey bee immune system is expansive using multiple pathways and processes to fight off pathogens, however social hymenoptera have been found to have fewer immune response genes than solitary insects (Barribeau et al., 2015; J. Evans et al., 2006). For bacterial and fungal infections, honey bee immune pathways include Toll, Imd, and JNK which are Nf-κB related pathways regulating Relish and Dorsal which are involved in effector gene transcription, as well as the JAK/STAT pathway which regulates synthesis of effector molecules like antimicrobial peptides (Evans, 2006; Kingsolver et al., 2013). For viral infections, immune pathways include siRNA/RNAi which limits and defends against virus infections, Toll, Imd, and JAK/STAT (Brutscher & Flenniken, 2015; Kingsolver et al., 2013). To evaluate immune response to viral exposure we wanted to select immune response genes from different immune pathways, therefore we selected *dicer-like* which is part of the RNAi pathway and *relish* which is part of the Imd pathway (Brutscher & Flenniken, 2015). Most of the knowledge on immune functions in bees have focused on bacteria, where researchers have demonstrated acquired immunity and found evidence of physiological mechanisms for transgenerational immune priming (Freitak et al,, 2009; Freitak et al., 2014; Moret, 2006; Roth et al., 2010; Sadd et al., 2005; Salmela et al., 2015; Zanchi et al., 2011).

Transgenerational immune priming is the process where offspring have increased resistance to infection due to parental immune experience being passed down to their progeny (Little et al., 2003). Despite previous thoughts that insects are not equipped with the immune machinery to have pathogen specificity or that they are capable of passing on any type of immune memory, transgenerational immune priming has been shown in some insects (Freitak et al., 2009; Freitak et al., 2014; Moret, 2006; Roth et al., 2010; Sadd et al., 2005; Salmela et al., 2015; Tidbury et al., 2010; Zanchi et al., 2011). Specifically for honey bees transgenerational immune priming has been shown for *Paenibacillus larvae,* a bacteria responsible for American Foulbrood, a deadly disease that decimates honey bee brood, and for *Escherichia coli* (López et al., 2014; Salmela et al., 2015). The physiological mechanism for bacterial transgenerational immune priming in honey bees has been explained, where bacteria are broken down by the immune system and bind to Vitellogenin (Vg) in the hemolymph which is then transported to developing (Salmela et al., 2015). It is hypothesized that Vg selectively binds to phosphatidylserine through charge-based affinity (Salmela et al., 2015). Vg is also known to experience rapid evolution in honey bees which could result in Vg variants with different pathogen specificity (Kent et al., 2011; Salmela et al., 2015). Although there is an increasing body of literature for immune priming for bacteria in insects, the relationship of insect-virus immune priming is not well understood. Previous research looked at a natural relationship between *Plodia interpunctella* (Lepidoptera) and Plodia interpunctella granulosis virus (PiGV), a DNA virus, as well as the relationship between *Drosophila melanogaster* and *Drosophila c virus* (DCV), a positive sense RNA virus (Longdon et al., 2013; Tidbury et al., 2010). Tidbury et al. (2010) found for within generation immune priming that exposure early on in life to a low dose of the virus led to reduced susceptibility during a second exposure to the virus at a lethal level. Tidbury et al. (2010) took the experiment even further by

testing transgenerational immune priming, where they found similar results where offspring of parents who faced a low dose challenge of the virus were less susceptible to the virus at a LD50 concentration (Tidbury et al., 2010). However, Longdon et al. (2013) did not find evidence for viral immune priming in *Drosophila*. Insect immune systems vary widely between orders, therefore the ability of honey bees to elicit an antiviral response through transgenerational immune priming should be further explored.

Given that *Deformed wing virus* (DWV) is the most prevalent honey bee virus (Tentcheva et al., 2004; Traynor et al., 2016), it provides an excellent opportunity to understand how immune responses, such as transgenerational immune priming, can be used to mitigate effects. DWV is not only capable of infecting honey bees at all life stages and all castes, but it can also be transmitted horizontally (worker-worker, worker-drone, worker-queen, drone-queen) and vertically (queen-offspring, drone-offspring). The virus has a nearly worldwide distribution and this global distribution is mirrored with the distribution of the virus's vector, *Varroa destructor*. Symptoms of DWV-A include deformed wings, decreased body size and weight, discoloration of adults, premature pupal death, and a severely reduced adult lifespan (De Miranda & Genersch, 2010). While DWV-A can be asymptomatic, it is a major threat to honey bees due to its high prevalence and ability to develop lethal symptoms in overt infections. The goal of this study was to explore the potential for transgenerational immune priming for DWV-A, a positive single stranded RNA virus, in honey bees, by inoculating queens orally and venereally and exposing their pupal offspring via microinjection to mimic vector transmission by *Varroa destructor.*

3.2. Materials and Methods

3.2.1. Insects

Honey bee queens were reared and maintained at the USDA ARS Honey Bee Breeding, Genetics, and Physiology research lab in Baton Rouge, LA following standard queen rearing procedures as described in chapter 2 in section 2.2.1 (Büchler et al., 2013). Queens exposed to DWV-A in this experiment were from two queen sources, Italian 202 and Italian 205, which queens within a queen source were sister queens.

3.2.2. Viral Homogenate Synthesis

The viral inoculum for queen treatments was prepared as described in chapter 2 section 2.2.3 using the same RNA extraction, cDNA synthesis, and qPCR screening processes. Viral homogenate synthesis for the pupae inoculum followed the same protocols but with the following modifications. During RNA extraction, bees were milled using an Omni (Kennesaw, GA) BeadRupter-24 Elite with a protocol of 2 cycles at 5m/s for 15s with a 10s dwell between cycles. 1.0mL of sterile 1X PBS was added to each sample and vortexed to settle precipitate. The samples were then centrifuged in an Eppendorf Centrifuge 5430 R at 4°C for 10 minutes at 14,000rpm. cDNA synthesis was conducted for 40µL reactions using 112.5ng/µL of RNA.

3.2.3. Queen Treatments

Queen treatments were conducted as described in chapter 2 section 2.2.3.

3.2.4. Experimental Sites

Experimental sites and set up is as described in chapter 2 section 2.2.5.1 and 2.2.5.2. White eye pupae (approximately 12 days after egg laying) were used for transgenerational immune priming testing and were collected from the colonies headed by the experimental queens at the end of the field season from October 3rd, 2018 through October 12th, 2018.

3.2.5. Pupae Treatment

White eye pupae were collected from 18 colonies (9 colonies per queen source, 3 colonies per queen transmission route) and were assigned to their treatment groups: no treatment (uninjected), injection with 3µL of sterile 1X PBS treatment (PBS), or injected with 3µL of DWV-A suspended in 1X PBS at a titer level of 10^7. This moderate dose was selected to minimize excessive pupal mortality that is observed during high titer injections and to ensure the virus is at a biologically relevant level, previous literature shows detection of the virus within *Varroa* mites from 10^4 to 10^{12} (Gisder, Aumeier, & Genersch, 2009). Microinjection was conducted using Hamilton (Reno NV) Gastight syringes fitted with Hamilton 1" 20° needles and World Precision Instruments (Sarasota, FL) SmartTouch Syringe Pump Controller Micro-2T. Following treatment, pupae were incubated at 34°C and 80% relative humidity until emergence (De Miranda et al., 2013). Pupae were checked daily to record mortality and emergence date. At 3 days post-treatment, 8 pupae per treatment group were stored at -80°C for molecular analysis. Upon emergence, DWV symptoms were recorded and 8 bees per treatment group were stored at -80°C for molecular analysis.

3.2.6. Viral and Antiviral Assessment of Pupae

Pupae were removed from -80°C storage for RNA extraction and 250µL of Promega homogenization solution and 250µL of Promega lysis buffer were added to each sample. Samples were then hand ground with a pestle, and then centrifuged in an Eppendorf 5430R centrifuge for 60 seconds at 14,000RPM at 4°C. RNA extraction followed the Maxwell® 16 LEV simplyRNA tissue protocol following the manufacturer's technical manual using 400µL of the sample homogenate (Promega, 2017). Upon completion of the "simplyRNA" program, the samples were stored at -80°C prior to NanoDrop One quantification.

cDNA synthesis follows the methods described in section 3.2.2 modified by using the Qiagen (Hilden, Germany) QuantiTect® Reverse Transcription Kit. cDNA synthesis followed the Qiagen QuantiTect® Reverse Transcription protocol for 20µL reactions using 30.0ng/µL of RNA for the cDNA synthesis reaction.

Pupae were analyzed using qPCR to quantify DWV-A, DWV-B, Ndufa38, *dicer-like*, and *relish*. Ndufa38 refers to a stable reference gene in honey bees named *NADH dehydrogenase (ubiquinone)* (Cameron et al., 2013). Two antiviral genes were selected for analysis, *dicer-like* and *relish*, due to their involvement in two different immune pathways that respond to virus infection, RNAi and Imd respectively (Brutscher et al., 2015). Recently, *relish* was implicated as a candidate for bacterial transgenerational immune priming in *Zootermopsis angusticollis*, a eusocial termite (Cole et al., 2020). DWV-A and DWV-B quantification was performed on the Applied Biosystems™ QuantStudio™ 6 Flex Real-Time PCR System with the PowerUp™ SYBR™ Green PCR Master Mix and relative quantification of *dicer-like* and *relish* in relation to the reference gene Ndufa38 was performed on the BioRad CFX Connect using BioRad SsoAdvanced™ Universal SYBR® Green Supermix using standard primer pairs (Table 3.2). Master mix configuration and qPCR protocol for DWV-A and DWV-B are described in section 3.2.2. BioRad CFX Connect reactions contained 5.0µL master mix, 3.0µL nuclease-free H_2O, 1.0µL cDNA template, and 0.5µL forward and reverse primers. BioRad CFX Connect thermal cycling protocol for Ndufa38, *dicer-like*, and *relish*: 5 min at 95°C, followed by 40 cycles of 95°C for 5s, 59.0°C for 10s, 72.0°C for 3s, and melt curve analysis for 65.0°C to 95.0°C for 5s.

Relative quantification of Dicer-like and Relish was determined by calculating the fold change in their expression using the $2^{-\Delta\Delta C}$t method (Livak & Schmittgen, 2001). Replicate runs of each sample were averaged and fold change was calculated within each queen source. The internal

control gene used to determine relative expression was Ndufa38. Since there was no time component, the target genes in treated pupae were compared to those in the untreated control pupae that were descended from untreated control queens.

3.2.7. Statistical Analysis

All statistical analyses were performed in JMP® Pro 14 (SAS Institute Inc, 2019). Analyses were carried out to determine if queen source, virus treatments for queens, and/or virus treatments for pupae had significant impact on the level of wing deformity, days to emergence, virus presence and titer for DWV-A, and DWV-B, and relative quantification of *dicer-like* and *relish*.

Percent mortality, days to emergence, log transformed viral titers, and log transformed $2^{-\Delta\Delta C_T}$ were analyzed by three way ANCOVA using least squares means model where we evaluated the main effects and interactions of the following variables: queen source, virus inoculation treatment of queen, injection treatment of pupae. We also included colony as a random effect. Post-hoc analyses consisted of least squares means differences with Tukey HSD. Wing deformity, detection of DWV-A, and detection of DWV-B were analyzed by logistic regression with using general linear models with binomial distribution where we evaluated the main effects and interactions between queen source, queen virus treatment, and pupae virus treatment. Post-hoc analyses of wing deformity, DWV-A, and DWV-B consisted of categorical response analysis and response homogeneity tests with Pearson's chi-square and Fisher's exact.

3.3. Results

Queens from Italian 202 and Italian 205 colonies which pupae were tested did not have detectable levels of DWV-A at the time of challenging pupae, 5 months after exposing queens with DWV-A through different transmission routes n = 21 colonies (11 colonies for Italian 202 and 10 colonies for Italian 205, 7 colonies per queen inoculation route across all queen sources)

(data not shown). There was no significant random effect on mortality due to colony, $p = 0.0643$, so colonies were combined for analysis. Virus treatment of pupae had a significant effect on percent morality, $F_2 = 3.791$ $p = 0.0385$, with PBS injected pupae having higher mortality than uninjected pupae and DWV-A injected pupae being intermediate but not significantly different from either treatment (figure 3.1). Overall, mortality was quite low at less than 15%. There was no significant effect due to queen source ($F_1 = 0.107$ $p = 0.7503$), queen DWV-A inoculation treatment ($F_2 = 0.503$ $p = 0.6178$), or any two-way or three-way interactions ($p > 0.1$ for all comparisons; table A.6).

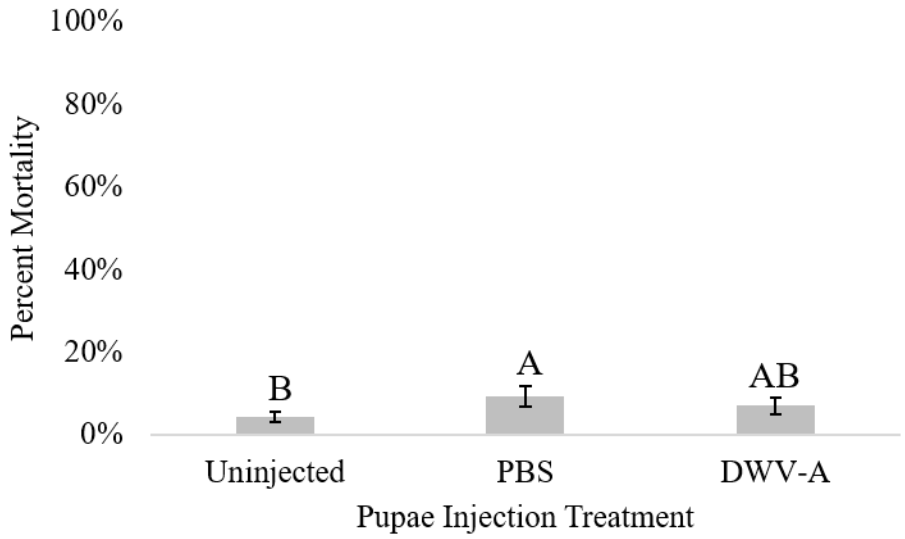

Figure 3.1. Percent morality of pupae by injection treatment n=17 colonies (an average of 697 individuals ± 40 per colony). Bars accompanied by the same letter represent no significance ($p > 0.05$; Tukey's HSD). Error bars represent one standard error from the mean.

For wing deformity, there was a significant interaction between queen source, queen DWV-A inoculation route, and pupae injection treatment ($\chi^2 = 13.968$, df = 1,2,2, $p = 0.0074$; figure 3.2). Wing deformity symptoms in pupae injected with DWV-A exhibited different patterns between the two queen sources in relation to the DWV-A exposure to queens. For Italian 202 queen source, pupal injection with DWV-A led to increased percentage of pupae developing wing deformities regardless of queen exposure (figure 3.2A). However for the Italian 205 queen source,

pupae reared from queens exposed to DWV-A, orally or venereally, had significantly more symptom development compared to pupae reared from non-inoculated queens (figure 3.2B).

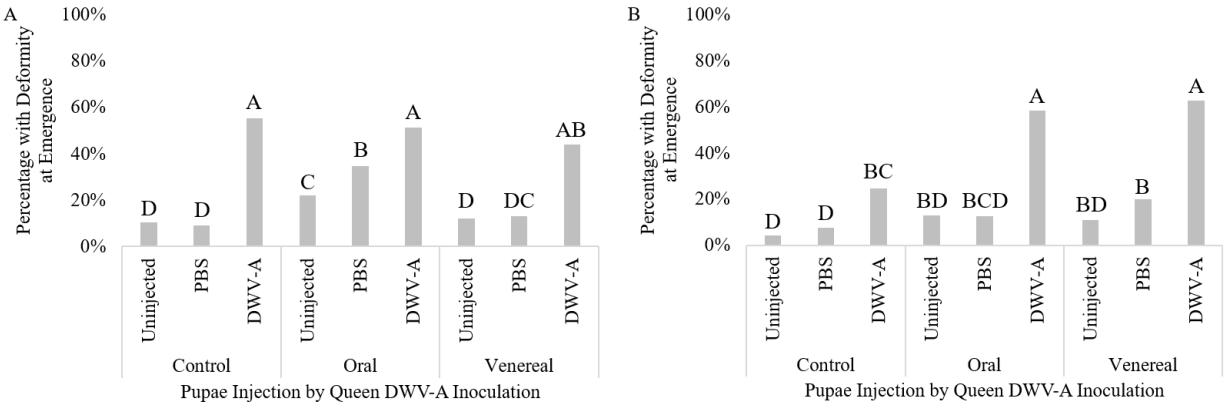

Figure 3.2. Percentage of pupae that emerged with wing deformities for each pupae injection treatment within each queen DWV-A inoculation route for (A) queen source Italian 202 n = 18 colonies (an average of 122 individuals ± 16 per colony), and (B) queen source Italian 205 n = 18 colonies (an average of 106 individuals ± 34 per colony). Bars accompanied by the same letter represent no significance ($p > 0.05$; Fisher's).

There was a random effect on days to emergence due to colony, $p = 0.0179$, likely due to slight genetic differences in development time or due to age of pupae sampled varying slightly. There was a significant interaction between queen source, queen DWV-A inoculation route, and pupae injection treatment ($F_{1,2,2} = 16.188$, $p < 0.0001$; figure 3.3). Overall Italian 202 pupae emerged earlier than Italian 205 pupae. Italian 202 DWV-A injected pupae emerged earlier compared to uninjected and PBS injected pupae from control and orally inoculated queens, but DWV-A injected pupae from venereally inoculated queens was earlier than uninjected pupae but similar to PBS injected pupae. Italian 205 pupae emergence time was significantly earlier in DWV-A injected pupae compared with control and PBS injected pupae from oral and venereal inoculated queens, and earlier for injected pupae compared to uninjected pupae from non-inoculated queens.

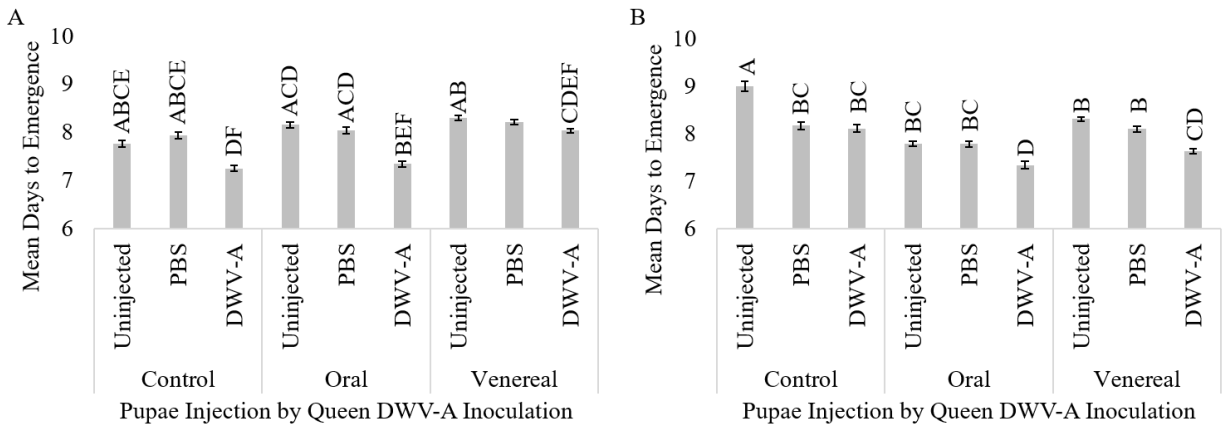

Figure 3.3. Mean days to emergence for pupae of different injection treatments within different queen DWV-A inoculation routes for (A) queen source Italian 202 n = 1,094 (n_{CC} = 151, n_{CPBS} = 123, n_{CDWV} = 125, n_{OC} = 127, n_{OPBS} = 107, n_{ODWV} = 109, n_{VC} = 139, n_{VPBS} = 106, n_{VDWV} = 107) and (B) queen source Italian 205 n = 954 (n_{CC} = 47, n_{CPBS} = 79, n_{CDWV} = 65, n_{OC} = 153, n_{OPBS} = 118, n_{ODWV} = 123, n_{VC} = 128, n_{VPBS} = 126, n_{VDWV} = 115). Bars accompanied by the same letter represent no significance ($p > 0.05$; Tukey HSD). Error bars represent one standard error from the mean.

The presence of DWV-A was significantly affected by pupae injection treatment, (χ^2 = 141.152, df = 2, $p < 0.0001$), with uninjected pupae (0.00%) and PBS injected pupae (1.85%) having a lower prevalence of DWV-A than DWV-A injected pupae (83.33%). The presence of DWV-A was not significantly affected by queen source ($\chi^2 = 2.938 \times 10^{-7}$, df = 1, $p = 0.9996$), queen DWV-A inoculation route ($\chi^2 = 1.762 \times 10^{-5}$, df = 2, $p = 1.0000$), or any two or three-way interactions ($p > 0.1$ for all comparisons; table A.7).

There was no random effect on DWV-A titer due to colony, $p = 0.9688$, or location, $p = 0.6146$. Virus titer of DWV-A was significantly affected by an interaction between queen source, queen DWV-A inoculation route, and pupae injection treatment ($F_{1,2,2} = 19.217$ $p < 0.0001$; figure 3.4). DWV-A titer in Italian 202 was lower in DWV-A injected pupae from venereally inoculated queens compared to DWV-A injected pupae from control and oral inoculated queens. DWV-A titer in Italian 205 was higher in DWV-A injected pupae from DWV-A exposed queens relative to pupae from non-inoculated queens, and had higher DWV-A titers than DWV-A injected pupae from venereal inoculated Italian 202 queens.

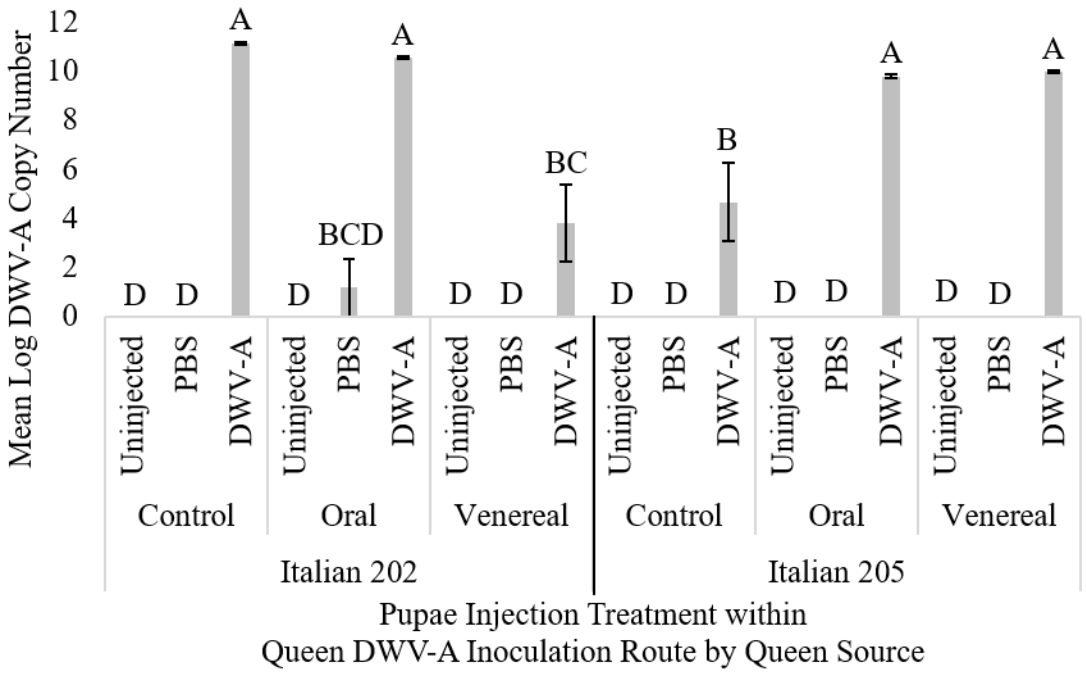

Figure 3.4. Mean log DWV-A copy number for pupae injections within queen DWV-A inoculation routes by queen source n = 162 (9 individuals per bar). Bars accompanied by the same letter represent no significance ($p > 0.05$; Tukey HSD). Error bars represent one standard error from the mean.

The presence of DWV-B was significantly affected by an interaction between queen source, queen DWV-A inoculation route, and pupae injection treatment ($\chi^2 = 10.443$, df = 1,2,2, $p = 0.0336$; figure 3.5A). DWV-B detection for Italian 202 was higher in DWV-A injected pupae across all queen treatments compared to Italian 205 DWV-A injected pupae from venereally inoculated queens and PBS injected pupae from orally inoculated queens. Overall, DWV-A injected pupae from Italian 205 did not significantly differ in DWV-B detection compared to uninjected and PBS injected pupae, while Italian 202 pupae injected with DWV-A had higher DWV-B detection compared to uninjected Italian 202 pupae. There was no random effect on DWV-A titer due to location, $p = 0.5687$, but there was a random effect due to colony, $p = 0.0.0285$. Log mean DWV-B virus titer was significantly affected by an interaction between queen source

and pupae injection treatment, $F_{1,2} = 8.402$, $p = 0.0004$, where DWV-B titer was higher in DWV-A injected pupae for Italian 202, but this was not seen for Italian 205 (figure 3.5B).

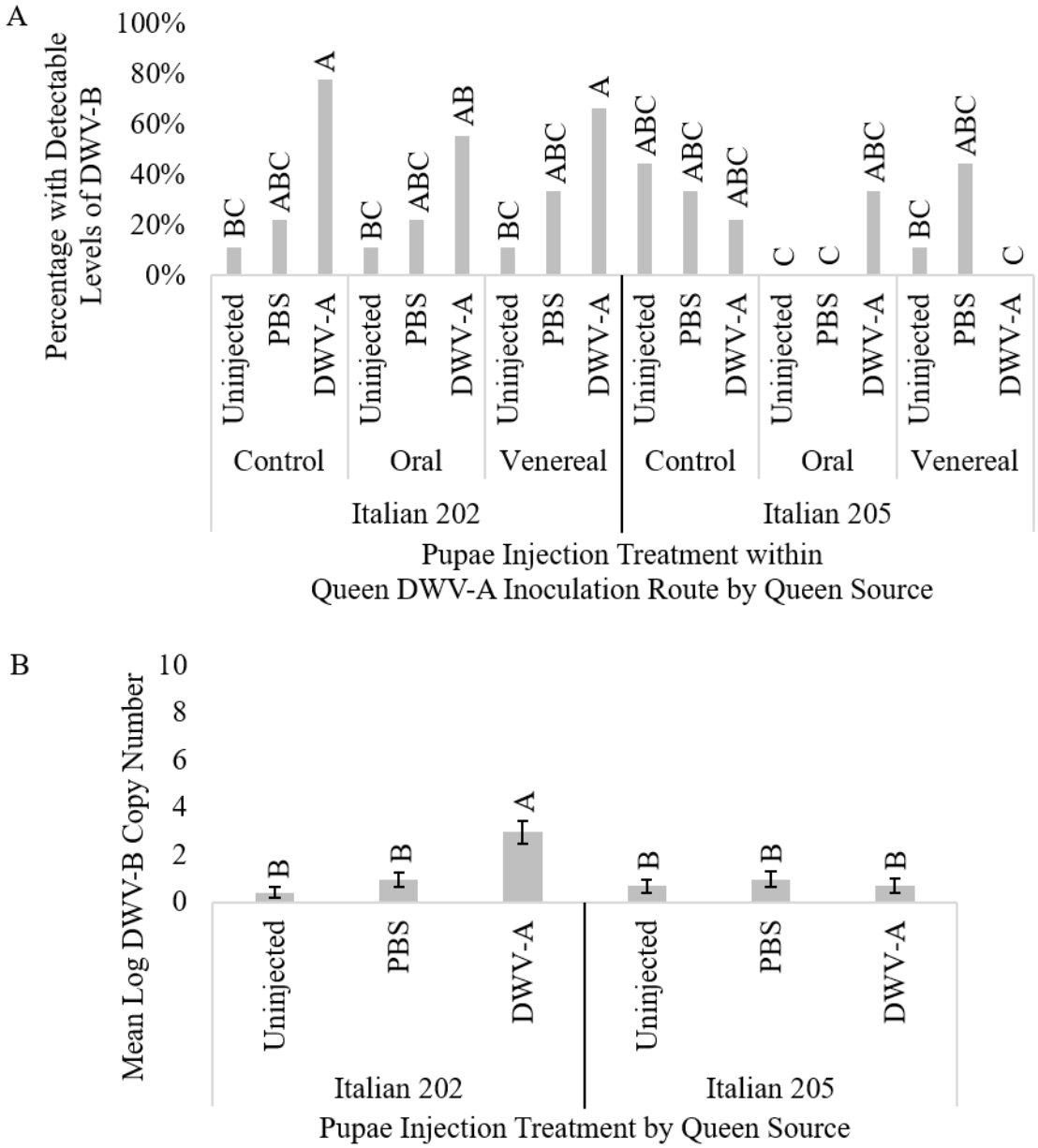

Figure 3.5. (A) Percentage of pupae with detectable levels of DWV-B n = 162 (9 individuals per bar) and (B) mean log DWV-B copy number for pupae injection treatments by queen source n = 162 (27 individuals per bar). Bars accompanied by the same letter represent no significance ($p > 0.05$; Tukey HSD). Error bars represent one standard error from the mean.

There were no random effects on relative quantity of *dicer-like* due to colony, $p = 0.1358$. The relative quantity of *dicer-like* was significantly affected by interactions between queen source,

queen DWV-A inoculation route, and pupae injection treatment ($F_{1,2,2} = 2.479$ $p = 0.0471$; figure 3.6). Italian 205 pupae from venereal inoculated queens had higher fold change of *dicer-like* expression compared with Italian 202 pupae and Italian 205 pupae from non-inoculated queens and oral inoculated queens. Italian 202 injected pupae from venereal inoculated queens had lower fold change of *dicer-like* expression compared with all groups within Italian 205 except for uninjected pupae from non-inoculated queens.

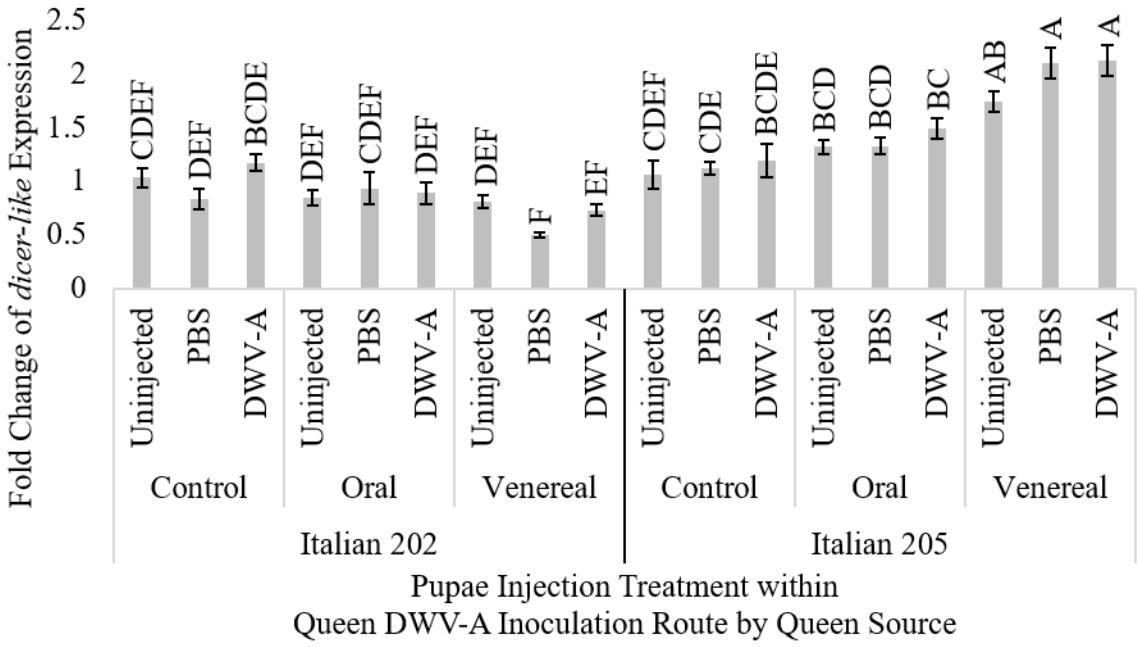

Figure 3.6. Fold change relative quantity of dicer-like for n = 162 (9 individuals per bar). Bars accompanied by the same letter represent no significance (p > 0.05; Tukey HSD). Error bars represent one standard error from the mean.

There were no random effects on relative quantity of *relish* due to colony, $p = 0.0655$. The relative quantity of *relish* was significantly affected by an interaction between queen source and queen DWV-A inoculation route ($F_{1,2} = 6.105$ $p = 0.0148$; figure 3.7) where pupae from Italian 202 venereal inoculated queens had a significantly lower fold change of *relish* than uninjected Italian 202 pupae and pupae from Italian 205 queens. There were no significant

effects due to pupae injection treatment ($F_2 = 1.320$ $p = 0.2706$) or interactions involving pupae injection treatment ($p > 0.1$ for all comparisons; table A.8)

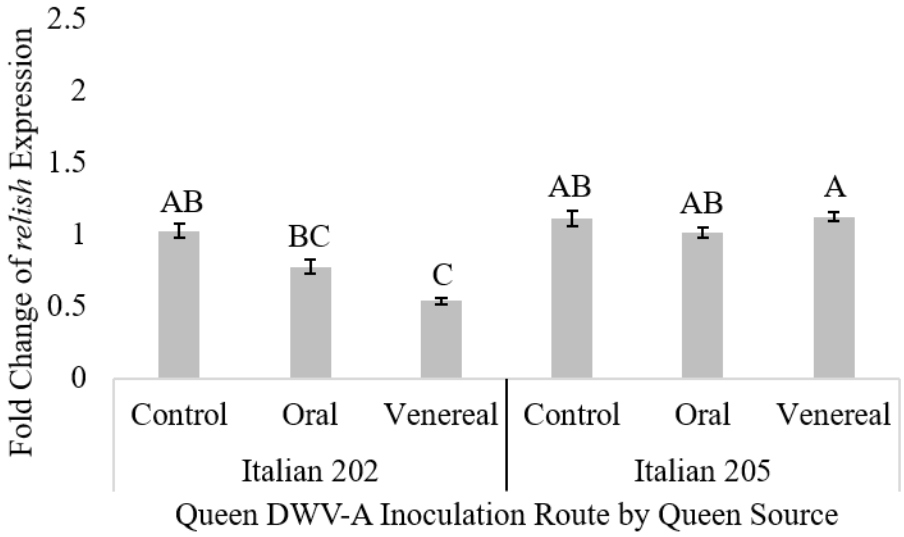

Figure 3.7. Fold change relative quantity of relish n = 162 (27 individuals per bar). Bars accompanied by the same letter represent no significance (p > 0.05; Tukey HSD). Error bars represent one standard error from the mean.

3.4. Discussion

Transgenerational immune priming has been demonstrated in honey bees for bacterial infections, however viral transgenerational immune priming has yet to be studied (López et al., 2014; Salmela et al., 2015). Our results suggest that there is evidence for viral transgenerational immune priming but expression of transgenerational immune priming is different between queen genotypes, and that venereally inoculating queens with DWV-A rather than oral inoculation has more transgenerational immune priming potential. We show that venereal exposure to DWV-A to queens in one queen genotype leads to a reduction of DWV-A and changes in expression of *dicer-like* and *relish* in pupae challenged with DWV-A injection. These findings demonstrate that transgenerational immune priming may be occurring in honey bees in response to viral exposure and that genetics may play a role in the expression of transgenerational immune priming.

We found queen sources differed in their expression of transgenerational immune priming, where queen source refers to the maternal origin of queens that were differentially exposed to virus and subsequently used to rear pupae for this experiment, and so represents two distinct queen genotypes in all tests. This suggests that there is a genetic component to viral transgenerational immune priming in honey bees. Our data also shows that for the Italian 202 queen source, the maternal genotype exhibited evidence for transgenerational immune priming. Venereal inoculation of queens led to more DWV-A resistance in offspring compared to queens that were orally inoculated. DWV-A injected pupae from venereally inoculated Italian 202 queens had lower DWV-A titer compared with DWV-A injected pupae from non-inoculated and orally inoculated Italian 202 queens as well as DWV-A injected pupae from oral and venereal inoculated Italian 205 queens. Within these same pupae from Italian 202 venereal inoculated queens we also found significantly lower fold change expression of *dicer-like* and *relish*, two genes involved in the honey bee immune pathways, RNAi and Imd respectively. Italian 205 did not show evidence for transgenerational immune priming. Two previous studies have assessed regulation of *dicer-like* in response to viral infections, the first study by Ryabov et al. (2014) found no change in *dicer-like* expression in pupae exposed to *Varroa* mites and high DWV levels, and the second by Galbraith et al. (2015) found upregulation of *dicer-like* in adult workers fed virus extracts from bees infected with IAPV, DWV, BQCV, KBV, and SBV (Galbraith et al., 2015; Ryabov et al., 2014). The differences in gene expression between our data and the previous research could be due to timing of sampling. Galbraith et al sampled the virus exposed workers at 24 hours after treatment, while in our study pupae were sampled 3 days after treatment. Galbraith et al. (2015) likely captured the upregulation of *dicer-like* due to the earlier sampling time, whereas our study captured the downregulation of *dicer-like* due to the later sampling time. The RNAi pathway may be short

acting and capturing upregulating transcriptome changes may only be possible at the beginning of pathogen exposure (Doublet et al., 2017). This is further supported by the virus detection and titer in DWV injected pupae from Italian 202 venereally inoculated queens (figure 3.5), where the virus detection and titer level were significantly lower indicating that antiviral immune response may be quicker acting than Italian 205 and have already responded to the virus infection, or another mechanism resulted in reduced viral replication and hence lower need immune expression. Previous research has shown evidence for variation in transgenerational immune priming between different colonies which could further support a genetic related variation of expression (López et al., 2014). Previous research has also shown genetic variation can influence the development of virus symptoms, virus titer, and antiviral response (Boncristiani et al., 2013; Khongphinitbunjong et al., 2015; Kulinčević & Rothenbuhler, 1975; Rinderer et al., 1975). An alternative mechanism for transgenerational immune priming could be differences in DNA methylation through epigenetic changes. These findings in addition to the data from our study suggests that transgenerational immune priming of viruses is possible in honey bees, but is controlled by genetics. This conclusion leads to the possibility that transgenerational immune priming could be selectively bred for if heritable, which could lead to increased virus resistance.

Injection with DWV-A significantly reduced emergence time in pupae and there was no difference in mortality across any of the pupae injection treatments. Previous research has shown that inducing immune response increased metabolic rate for multiple insect species, which may also be a consequence of virus injection for honey bees (Ardia et al., 2012). The consequences of accelerated pupal development in honey bees is unknown. Accelerated pupal development could be beneficial for the virus in terms of disease spread, with workers emerging earlier, the brood cell is opened enabling *Varroa* mites to find new hosts and spread DWV to developing brood and nurse

bees, leading to acceleration of virus spread throughout the colony. This can be further supported by our data showing no difference in mortality between uninjected, PBS injected, and DWV-A injected pupae. This means that pupae injected with DWV-A emerge successfully and are able to further transmit the virus through oral transmission while performing nurse duties. Alternatively this could reduce *Varroa* populations within a colony by shortening the available time for *Varroa* reproduction and development of their offspring. This is seen in Africanized honey bees which develop more quickly than non-Africanized honey bees, which is a known mechanism of resistance to *Varroa* and the pathogens they transmit (Camazine, 1986). Therefore, although reduced development time was an unexpected finding, it may play a role in the epidemiology and the relationship between honey bees and the virus. DWV has already been implicated in negative effects on worker health and foraging capabilities, however whether or not accelerated development time impacts these has not been studied and is unknown (Benaets et al., 2017; Iqbal & Mueller, 2007; Yang & Cox-Foster, 2005).

The presence and titer of DWV-B in pupae was lower in pupae injected with PBS compared to pupae injected with DWV-A and uninjected pupae, which were not statistically different from each other. This may be due to an upregulation in immune response caused by trauma from injection with PBS, which isn't seen in the DWV-A injected group due to DWV-A suppressing the immune system of its host (Lourenço et al., 2013; Nazzi et al., 2012). Previous research shows that DWV variants A and B demonstrate mutual compatibility and lack competitive exclusion during coinfection, which explains finding DWV-A and DWV-B cohabiting pupae (Ryabov et al., 2019).

In summary, there is evidence that viral transgenerational immune priming may be occurring in honey bees dependent on genetics when queens are venereally exposed to virus rather

than oral exposure. Queens in this experiment were single exposed to DWV-A, multiple exposures to the virus may have different effects especially in regards to oral transmission since the queen is fed by workers which allows for oral transmission of pathogens multiple times over the lifespan of the queen. We also found that injection with DWV-A reduces emergence time, did not affect mortality, and that there may be a mutualistic compatibility between DWV variants in this experiment. These results show evidence that there is a genetic basis for viral transgenerational immune priming. Further research must be conducted to determine heritability of this potential trait and if it could in turn be selectively bred for to produce DWV resistant genotypes. It is unknown the potential mechanism for viral transgenerational immune priming, however it seems unlikely to utilize vitellogenin since the vitellogenin pathogen binding pattern is predominantly a gram-positive bacteria signature (Salmela et al., 2015). However, vitellogenin is believed to have rapidly evolved in honey bees which may allow for different vitellogenin variants that are able to bind to a wide array of pathogens (Kent et al., 2011; Salmela et al., 2015). Future studies should further test for viral transgenerational immune priming with more queen genotypes to determine how regularly it is observed, if it is seen for specific viruses, and how beneficial it could be. Although DWV-A is capable of multiple strategies for survival and transmission, viral transgenerational immune priming could be a defense against DWV and reduce colony loss.

3.5. References

Ardia, D. R., Gantz, J. E., & Strebel, S. (2012). Costs of immunity in insects: an induced immune response increases metabolic rate and decreases antimicrobial activity. *Functional Ecology*, *26*(3), 732-739.

Barribeau, S. M., Sadd, B. M., du Plessis, L., Brown, M. J., Buechel, S. D., Cappelle, K., . . . Erler, S. (2015). A depauperate immune repertoire precedes evolution of sociality in bees. *Genome biology*, *16*(1), 83.

Benaets, K., Van Geystelen, A., Cardoen, D., De Smet, L., de Graaf, D. C., Schoofs, L., . . . Wenseleers, T. (2017). *Covert deformed wing virus infections have long-term deleterious effects on honeybee foraging and survival.* Paper presented at the Proc. R. Soc. B.

Boncristiani, H. F., Evans, J. D., Chen, Y., Pettis, J., Murphy, C., Lopez, D. L., . . . Rueppell, O. (2013). In vitro infection of pupae with Israeli acute paralysis virus suggests disturbance of transcriptional homeostasis in honey bees (Apis mellifera). *PLOS One, 8*(9), e73429.

Brutscher, L. M., Daughenbaugh, K. F., & Flenniken, M. L. (2015). Antiviral defense mechanisms in honey bees. *Current Opinion in Insect Science, 10*, 71-82.

Brutscher, L. M., & Flenniken, M. L. (2015). RNAi and antiviral defense in the honey bee. *Journal of immunology research, 2015*.

Büchler, R., Andonov, S., Bienefeld, K., Costa, C., Hatjina, F., Kezic, N., . . . Wilde, J. (2013). Standard methods for rearing and selection of Apis mellifera queens. *Journal of Apicultural Research, 52*(1), 1-30.

Camazine, S. (1986). Differential reproduction of the mite, Varroa jacobsoni (Mesostigmata: Varroidae), on Africanized and European honey bees (Hymenoptera: Apidae). *Annals of the Entomological Society of America, 79*(5), 801-803.

Cameron, R. C., Duncan, E. J., & Dearden, P. K. (2013). Stable reference genes for the measurement of transcript abundance during larval caste development in the honeybee. *Apidologie, 44*(4), 357-366.

Cole, E. L., Empringham, J. S., Biro, C., Thompson, G. J., & Rosengaus, R. B. (2020). Relish as a Candidate Marker for Transgenerational Immune Priming in a Dampwood Termite (Blattodae: Archeotermopsidae). *Insects, 11*(3), 149.

De Miranda, J. R., Bailey, L., Ball, B. V., Blanchard, P., Budge, G. E., Chejanovsky, N., . . . De Graaf, D. C. (2013). Standard methods for virus research in Apis mellifera. *Journal of Apicultural Research, 52*(4), 1-56.

De Miranda, J. R., & Genersch, E. (2010). Deformed wing virus. *Journal of Invertebrate Pathology, 103*, S48-S61.

Doublet, V., Poeschl, Y., Gogol-Döring, A., Alaux, C., Annoscia, D., Aurori, C., . . . Bull, J. C. (2017). Unity in defence: honeybee workers exhibit conserved molecular responses to diverse pathogens. *BMC Genomics, 18*(1), 207.

Evans, J., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J. L., Jiang, H., . . . Hultmark, D. (2006). Immune pathways and defence mechanisms in honey bees Apis mellifera. *Insect Molecular Biology, 15*(5), 645-656.

Evans, J. D. (2006). Beepath: an ordered quantitative-PCR array for exploring honey bee immunity and disease. *Journal of Invertebrate Pathology, 93*(2), 135-139.

Freitak, D., Heckel, D. G., & Vogel, H. (2009). Dietary-dependent trans-generational immune priming in an insect herbivore. *Proceedings of the Royal Society of London B: Biological Sciences, 276*(1667), 2617-2624.

Freitak, D., Schmidtberg, H., Dickel, F., Lochnit, G., Vogel, H., & Vilcinskas, A. (2014). The maternal transfer of bacteria can mediate trans-generational immune priming in insects. *Virulence, 5*(4), 547-554.

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.

Gisder, S., Aumeier, P., & Genersch, E. (2009). Deformed wing virus: replication and viral load in mites (Varroa destructor). *Journal of General Virology, 90*(2), 463-467.

Iqbal, J., & Mueller, U. (2007). Virus infection causes specific learning deficits in honeybee foragers. *Proceedings of the Royal Society B: Biological Sciences, 274*(1617), 1517-1521.

Kent, C. F., Issa, A., Bunting, A. C., & Zayed, A. (2011). Adaptive evolution of a key gene affecting queen and worker traits in the honey bee, Apis mellifera. *Molecular Ecology, 20*(24), 5226-5235.

Khongphinitbunjong, K., de Guzman, L. I., Tarver, M. R., Rinderer, T. E., Chen, Y., & Chantawannakul, P. (2015). Differential viral levels and immune gene expression in three stocks of Apis mellifera induced by different numbers of Varroa destructor. *Journal of insect physiology, 72*, 28-34.

Kingsolver, M. B., Huang, Z., & Hardy, R. W. (2013). Insect antiviral innate immunity: pathways, effectors, and connections. *Journal of Molecular Biology, 425*(24), 4921-4936.

Kulinčević, J. M., & Rothenbuhler, W. C. (1975). Selection for resistance and susceptibility to hairless-black syndrome in the honeybee. *Journal of Invertebrate Pathology, 25*(3), 289-295.

Little, T. J., O'Connor, B., Colegrave, N., Watt, K., & Read, A. F. (2003). Maternal transfer of strain-specific immunity in an invertebrate. *Current Biology, 13*(6), 489-492.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods, 25*(4), 402-408.

Longdon, B., Cao, C., Martinez, J., & Jiggins, F. M. (2013). Previous exposure to an RNA virus does not protect against subsequent infection in Drosophila melanogaster. *PLOS One, 8*(9), e73833.

López, J. H., Schuehly, W., Crailsheim, K., & Riessberger-Gallé, U. (2014). Trans-generational immune priming in honeybees. *Proceedings of the Royal Society B, 281*(1785), 20140454.

Lourenço, A. P., Guidugli-Lazzarini, K. R., Freitas, F. C., Bitondi, M. M., & Simões, Z. L. (2013). Bacterial infection activates the immune system response and dysregulates microRNA expression in honey bees. *Insect Biochemistry and Molecular Biology, 43*(5), 474-482.

Moret, Y. (2006). 'Trans-generational immune priming': specific enhancement of the antimicrobial immune response in the mealworm beetle, Tenebrio molitor. *Proceedings of the Royal Society of London B: Biological Sciences, 273*(1592), 1399-1405.

Naug, D., & Camazine, S. (2002). The role of colony organization on pathogen transmission in social insects. *Journal of Theoretical Biology, 215*(4), 427-439.

Nazzi, F., Brown, S. P., Annoscia, D., Del Piccolo, F., Di Prisco, G., Varricchio, P., . . . Pennacchio, F. (2012). Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. *PLoS Pathogens, 8*(6), e1002735.

Promega. (2017). *Maxwell 16 LEV simplyRNA Tissue Kity: Technical Manual*

Rinderer, T. E., Rothenbuhler, W. C., & Kulinčević, J. M. (1975). Responses of three genetically different stocks of the honeybee to a virus from bees with hairless-black syndrome. *Journal of Invertebrate Pathology, 25*(3), 297-300.

Roth, O., Joop, G., Eggert, H., Hilbert, J., Daniel, J., Schmid-Hempel, P., & Kurtz, J. (2010). Paternally derived immune priming for offspring in the red flour beetle, Tribolium castaneum. *Journal of Animal Ecology, 79*(2), 403-413.

Ryabov, E. V., Childers, A. K., Lopez, D., Grubbs, K., Posada-Florez, F., Weaver, D., . . . Evans, J. D. (2019). Dynamic evolution in the key honey bee pathogen Deformed wing virus. *PLoS Biology*, 653543.

Ryabov, E. V., Wood, G. R., Fannon, J. M., Moore, J. D., Bull, J. C., Chandler, D., . . . Evans, D. J. (2014). A virulent strain of Deformed wing virus (DWV) of honeybees (Apis mellifera) prevails after Varroa destructor-mediated, or in vitro, transmission. *PLoS Pathogens, 10*(6), e1004230.

Sadd, B. M., Kleinlogel, Y., Schmid-Hempel, R., & Schmid-Hempel, P. (2005). Trans-generational immune priming in a social insect. *Biology Letters, 1*(4), 386-388.

Salmela, H., Amdam, G. V., & Freitak, D. (2015). Transfer of immunity from mother to offspring is mediated via egg-yolk protein vitellogenin. *PLoS Pathogens, 11*(7), e1005015.

SAS Institute Inc. (2019). JMP® Pro (Version 14). Cary, NC.

Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M. E., & Bergoin, M. (2004). Prevalence and seasonal variations of six bee viruses in Apis mellifera L. and Varroa destructor mite populations in France. *Applied and Environmental Microbiology, 70*(12), 7185-7191.

Tidbury, H. J., Pedersen, A. B., & Boots, M. (2010). Within and transgenerational immune priming in an insect to a DNA virus. *Proceedings of the Royal Society B: Biological Sciences, 278*(1707), 871-876.

Traynor, K. S., Rennich, K., Forsgren, E., Rose, R., Pettis, J., Kunkel, G., . . . Lopez, D. (2016). Multiyear survey targeting disease incidence in US honey bees. *Apidologie, 47*(3), 325-347.

Yang, X., & Cox-Foster, D. L. (2005). Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host immunosuppression and viral amplification. *Proceedings of the National Academy of Sciences of the United States of America, 102*(21), 7470-7475.

Zanchi, C., Troussard, J. P., Martinaud, G., Moreau, J., & Moret, Y. (2011). Differential expression and costs between maternally and paternally derived immune priming for offspring in an insect. *Journal of Animal Ecology, 80*(6), 1174-1183.

SUMMARY AND CONCLUSION

Honey bees are important agricultural pollinators necessary for the production of many of our foods. However, managed honey bee colonies have been experiencing high levels of colony loss over the last decade due to a multitude of different factors. Parasites, pathogens, and queen failure is repeatedly reported as major causes for colony loss, but there is little research exploring the relationship between honey bee viruses and queens. *Deformed wing virus* (DWV), a major honey bee virus, has a worldwide distribution, is extremely prevalent in honey bee colonies, and can infect all castes and life stages of honey bees. Honey bee queens can be infected with the virus through multiple transmission routes and can be a major source of disease spread within a colony due to close contact with workers in addition to vertical transmission to the eggs she lays. It is important to understand how route of virus transmission impacts the establishment and intensity of virus infection in queens, as well as how this virus exposure could potentially impact colony strength and health. Therefore, the focus of this thesis research was to evaluate if route of DWV transmission led to differential virus infection and intensity in honey bee queens, effected colony strength and brood health, and if transgenerational immune priming for viruses is occurring in honey bees.

To evaluate if route of DWV transmission led to differential virus infection and intensity in honey bee queens, we artificially inoculated queens through oral transmission, venereal transmission, and a combination of both transmission routes and assessed virus presence and titer over time. We found that artificial inoculation with DWV-A through oral and venereal transmission was successful in delivering the virus to the queens, but none of the routes tested led to established high virus titer infections which suggests that honey bee queens are capable of preventing virus infection potentially through purging seminal fluid for venereal transmission and

physiological barriers within the gut lining preventing pathogen movement into the body for oral transmission. We concluded that single exposure through oral and venereal transmission may not be a significant threat to honey bee queen health and survival.

To assess colony strength and brood health following queen exposure to DWV, we artificially inoculated queens with DWV through oral transmission and venereal transmission, placed the exposed queens into colonies in the field, and collected colony assessment metrics every six weeks and sampled eggs and pupae for viral analysis. Queen source exhibited differential colony survival in response to the different DWV transmission routes, with colonies from one queen source showing higher rates of colony loss for DWV inoculated queens. Queen sources can differ in genetic background which can affect resistance to pathogens (Boncristiani et al., 2013; Khongphinitbunjong et al., 2015; Niño & Jasper, 2015). They can also differ in pathogen exposure potentially making the reared queens more susceptible to stressors and other viruses if the colony they were sourced from had high virus infections or multiple viruses present since vertical transmission is possible for some honey bee viruses (Ravoet et al., 2015). These two differences between queen sources could cause differential colony survival outcomes due to DWV-A inoculation since one queen source may be more vulnerable and susceptible to virus infection due to suppressed immune response or synergistic effects between multiple pathogens and other stressors (Nazzi et al., 2012; Yang & Cox-Foster, 2005; Zheng et al., 2015). Of the colonies that survived the summer, colonies headed by experimentally inoculated queens were not negatively impacted as far as colony strength parameters or disease development in brood, however queens in this study were single exposed to DWV-A and the effects of multiple exposures to DWV-A is unknown. We conclude that exposure to DWV can result in colony loss for some queen sources

that may be more vulnerable, but if the queen survives the inoculation the colony's strength and health is not negatively impacted.

To assess whether transgenerational immune priming for viruses was occurring in honey bees we injected pupae from artificially DWV inoculated queens with DWV and collected mortality, symptom development, emergence time, virus presence and titer, and antiviral immune response data. We found evidence that transgenerational immune priming may be occurring in venereal exposed queens and expression of transgenerational immune priming may differ based on genetic differences between queen sources. However, further research is needed to further confirm the occurrence of transgenerational immune priming.

The threat of colony loss is a major concern not only for beekeepers but for farmers who rely on honey bee pollination. Although there are many factors at play when it comes to colony loss, pathogens play a serious role due to their abundance and devastating effects on the health of a colony. This research shows that DWV-A inoculation of honey bee queens does not negatively impact queens or the colonies headed by these queens unless they are of a susceptible genotype, and that maternal virus experience may benefit offspring challenged by the same virus. This research also demonstrates how genetics plays a significant role in honey bee health, which demonstrates the importance for beekeepers to value genetic diversity between their colonies in an effort to minimize colony losses.

APPENDIX

Table A.1. Model effect statistical results for DWV-A detection, DWV-A titer, DWV-B detection, and DWV-B titer for queens exposed to DWV-A through different inoculation routes. Time: time after inoculation, VI: DWV-A inoculation route for queens.

		DWV-A Detection		**DWV-A Titer**		**DWV-B Detection**		**DWV-B Titer**	
Effect Source	**Degrees of Freedom**	χ^2	***p*-value**	**F Ratio**	***p*-value**	χ^2	***p*-value**	**F Ratio**	***p*-value**
Time	3	28.4946	**<0.0001**	4.4726	**0.0063**	3.3057	0.3469	1.8359	0.1480
VI	3	11.6739	**0.0086**	2.6821	0.0527	7.7775	0.0508	3.0168	**0.0350**
Time*VI	3,3	21.6844	**0.0099**	2.3548	**0.0211**	6.9945	0.6377	0.6606	0.7416

Table A.2. Model effect statistical results for BQCV detection, BQCV titer, CBPV detection, and CBPV titer for queens exposed to DWV-A through different inoculation routes. Time: time after inoculation, VI: DWV-A inoculation route for queens.

		BQCV Detection		**BQCV Titer**		**CBPV Detection**		**CBPV Titer**	
Effect Source	**Degrees of Freedom**	χ^2	***p*-value**	**F Ratio**	***p*-value**	χ^2	***p*-value**	**F Ratio**	***p*-value**
Time	3	29.3491	**<0.0001**	9.8436	**<0.0001**	0.5669	0.9040	0.2924	0.8308
VI	3	2.6949×10^{-8}	1.0000	0.7470	0.5274	7.3559	0.0614	2.3982	0.0744
Time*VI	3,3	0.7156	0.9999	0.5072	0.8651	12.4280	0.1902	1.2950	0.2535

Table A.3. Model effect statistical results for BQCV titer after five months for queens exposed to DWV-A through different inoculation routes. QS: queen source, VI: DWV-A inoculation route for queens, Tissue: tissue type.

		BQCV Titer	
Effect Source	**Degrees of Freedom**	**F Ratio**	***p*-value**
QS	1	0.0415	0.8416
VI	2	0.5159	0.6077
Tissue	3	58.4258	**<0.0001**
QS*VI	1,2	2.2928	0.1797
QS*Tissue	1,3	0.5965	0.6206
VI*Tissue	2,3	1.0763	0.3909
QS*VI*Tissue	1,2,3	1.4888	0.2037

Table A.4. Model effect statistical results for frames of bees, frames of brood, frames of pollen, and frames of nectar for colonies with queens exposed to DWV-A through different inoculation routes. QS: queen source, VI: DWV-A inoculation route for queens, Time: time after inoculation.

		Frames of Bees		**Frames of Brood**		**Frames of Pollen**		**Frames of Nectar**	
Effect Source	**Degrees of Freedom**	**F Ratio**	***p*-value**	**F Ratio**	***p*-value**	**F Ratio**	***p*-value**	**F Ratio**	***p*-value**
QS	1	0.1420	0.7082	0.6752	0.4164	0.3165	0.5780	0.0094	0.9233
VI	2	3.2455	**0.0491**	0.4796	0.6228	1.7166	0.1974	1.7222	0.1964
Time	2	26.9818	**<0.0001**	31.2509	**<0.0001**	6.3438	**0.0053**	39.4440	**<0.0001**
QS*VI	1,2	2.4218	0.1009	3.9429	**0.0286**	1.5863	0.2219	1.9435	0.1614
QS*Time	1,2	0.8921	0.4146	0.8964	0.4131	1.4845	0.2439	4.5595	**0.0193**
VI*Time	2,2	1.6768	0.1657	3.3755	**0.0144**	0.9695	0.4315	1.2117	0.3161
QS*VI*Time	1,2,2	0.6992	0.5952	0.6335	0.6404	0.9557	0.4390	2.2411	0.0761

Table A.5. Model effect statistical results for DWV-B detection, DWV-B titer, BQCV detection, and BQCV titer for eggs from queens exposed to DWV-A through different inoculation routes. QS: queen source, VI: DWV-A inoculation route for queens.

		DWV-B Detection		**DWV-B Titer**		**BQCV Detection**		**BQCV Titer**	
Effect Source	**Degrees of Freedom**	**χ^2**	***p*-value**	**F Ratio**	***p*-value**	**χ^2**	***p*-value**	**F Ratio**	***p*-value**
QS	1	5.8183×10^{-7}	0.9994	1.6167	0.2111	2.3877	0.1223	0.3244	0.5722
VI	2	3.5864×10^{-6}	1.0000	1.2158	0.3075	17.6326	**0.0001**	9.4979	**0.0004**
QS*VI	1,2	4.0353×10^{-6}	1.0000	0.3805	0.6860	5.3445	0.0691	1.5132	0.2329

Table A.6. Model effect statistical results for mortality, wing deformity, and days to emergence for DWV-A challenged pupae from queens exposed to DWV-A through different inoculation routes. QS: queen source, VI: DWV-A inoculation route for queens, VP: virus treatment of pupae.

		Mortality		**Wing Deformity**		**Days to Emergence**	
Effect Source	**Degrees of Freedom**	**F Ratio**	***p*-value**	χ^2	***p*-value**	**F Ratio**	***p*-value**
QS	1	0.1065	0.7503	5.9554	**0.0147**	0.7057	0.4171
VI	2	0.5033	0.6178	31.1549	**<0.0001**	2.4757	0.1253
VP	2	3.7910	**0.0385**	218.0321	**<0.0001**	149.9732	**<0.0001**
QS*VI	1,2	2.5905	0.1197	16.0486	**0.0003**	5.0094	**0.0259**
QS*VP	1,2	2.1907	0.1356	2.2355	0.3270	11.9175	**<0.0001**
VI*VP	2,2	2.1462	0.1090	2.7785	0.5956	3.4766	**0.0077**
QS*VI*VP	1,2,2	1.6836	0.1896	13.9685	**0.0074**	16.1867	**<0.0001**

Table A.7. Model effect statistical results for DWV-A detection, DWV-A titer, DWV-B detection, DWV-B titer for DWV-A challenged pupae from queens exposed to DWV-A through different inoculation routes. QS: queen source, VI: DWV-A inoculation route for queens, VP: virus treatment of pupae.

		DWV-A Detection		**DWV-A Titer**		**DWV-B Detection**		**DWV-B Titer**	
Effect Source	**Degrees of Freedom**	χ^2	***p*-value**	**F Ratio**	***p*-value**	χ^2	***p*-value**	**F Ratio**	***p*-value**
QS	1	2.9377×10^{-7}	0.9996	1.5543	0.2368	6.7691	**0.0093**	13.6278	**0.0033**
VI	2	1.7623×10^{-5}	1.0000	7.6107	**0.0077**	6.3072	**0.0427**	0.1563	0.8571
VP	2	141.1516	**<0.0001**	386.8504	**<0.0001**	1.3339×10^{-5}	1.0000	7.5521	**0.0008**
QS*VI	1,2	7.3910×10^{-6}	1.0000	13.7389	**0.0010**	4.5332	0.1037	3.6725	0.0583
QS*VP	1,2	8.7755×10^{-7}	1.0000	0.2030	0.8165	2.0342×10^{-5}	1.0000	8.4020	**0.0004**
VI*VP	2,2	5.0378×10^{-6}	1.0000	4.5239	**0.0019**	9.5308	**0.0491**	1.8648	0.1204
QS*VI*VP	1,2,2	2.4780×10^{-4}	1.0000	19.2168	**<0.0001**	10.4432	**0.0336**	0.5463	0.7020

Table A.8. Model effect statistical results for relative quantity of dicer-like and relish for DWV-A challenged pupae from queens exposed to DWV-A through different inoculation routes. QS: queen source, VI: DWV-A inoculation route for queens, VP: virus treatment of pupae.

		Relative Quantity of *dicer-like*		**Relative Quantity of *relish***	
Effect Source	**Degrees of Freedom**	**F Ratio**	***p*-value**	**F Ratio**	***p*-value**
QS	1	79.7564	**<0.0001**	25.8758	**0.0003**
VI	2	5.0229	**0.0260**	5.7395	**0.0178**
VP	2	3.9500	**0.0216**	1.3201	0.2706
QS*VI	1,2	24.6469	**<0.0001**	6.1047	**0.0148**
QS*VP	1,2	3.5691	**0.0309**	0.8477	0.4307
VI*VP	2,2	0.3587	0.8376	1.0002	0.4100
QS*VI*VP	1,2,2	2.4789	**0.0471**	1.3526	0.2539

REFERENCES

Aizen, M. A., & Harder, L. D. (2009). The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Current Biology, 19*(11), 915-918.

Amiri, E., Kryger, P., Meixner, M. D., Strand, M. K., Tarpy, D. R., & Rueppell, O. (2018). Quantitative patterns of vertical transmission of deformed wing virus in honey bees. *PLOS One, 13*(3), e0195283.

Amiri, E., Meixner, M. D., & Kryger, P. (2016). Deformed wing virus can be transmitted during natural mating in honey bees and infect the queens. *Scientific Reports, 6*.

Amiri, E., Strand, M. K., Rueppell, O., & Tarpy, D. R. (2017). Queen Quality and the Impact of Honey Bee Diseases on Queen Health: Potential for Interactions between Two Major Threats to Colony Health. *Insects, 8*(2), 48.

Ardia, D. R., Gantz, J. E., & Strebel, S. (2012). Costs of immunity in insects: an induced immune response increases metabolic rate and decreases antimicrobial activity. *Functional Ecology, 26*(3), 732-739.

Bakonyi, T., Farkas, R., Szendröi, A., Dobos-Kovács, M., & Rusvai, M. (2002). Detection of acute bee paralysis virus by RT-PCR in honey bee and Varroa destructor field samples: rapid screening of representative Hungarian apiaries. *Apidologie, 33*(1), 63-74.

Ball, B. (1983). *Association of Varroa jacobsoni with virus diseases of honey bees.* Varroa jacobsoni Oud. affecting honey bees: present status and needs: proceedings of a meeting of the EC Experts' Group.

Ball, B., & Allen, M. (1988). The prevalence of pathogens in honey bee (Apis mellifera) colonies infested with the parasitic mite Varroa jacobsoni. *Annals of Applied Biology, 113*(2), 237-244.

Barribeau, S. M., Sadd, B. M., du Plessis, L., Brown, M. J., Buechel, S. D., Cappelle, K., . . . Erler, S. (2015). A depauperate immune repertoire precedes evolution of sociality in bees. *Genome Biology, 16*(1), 83.

Benaets, K., Van Geystelen, A., Cardoen, D., De Smet, L., de Graaf, D. C., Schoofs, L., . . . Wenseleers, T. (2017). *Covert deformed wing virus infections have long-term deleterious effects on honeybee foraging and survival.* Paper presented at the Proc. R. Soc. B.

Blanchard, P., Ribière, M., Celle, O., Lallemand, P., Schurr, F., Olivier, V., . . . Faucon, J. P. (2007). Evaluation of a real-time two-step RT-PCR assay for quantitation of Chronic bee paralysis virus (CBPV) genome in experimentally-infected bee tissues and in life stages of a symptomatic colony. *Journal of Virological Methods, 141*(1), 7-13.

Boncristiani, H. F., Evans, J. D., Chen, Y., Pettis, J., Murphy, C., Lopez, D. L., . . . Rueppell, O. (2013). In vitro infection of pupae with Israeli acute paralysis virus suggests disturbance of transcriptional homeostasis in honey bees (Apis mellifera). *PLOS One, 8*(9), e73429.

Bowen-Walker, P., Martin, S., & Gunn, A. (1999). The transmission of Deformed wing virus between honeybees (Apis mellifera L.) by the ectoparasitic mite Varroa jacobsoni Oud. *Journal of Invertebrate Pathology, 73*(1), 101-106.

Brutscher, L. M., Daughenbaugh, K. F., & Flenniken, M. L. (2015). Antiviral defense mechanisms in honey bees. *Current Opinion in Insect Science, 10*, 71-82.

Brutscher, L. M., & Flenniken, M. L. (2015). RNAi and antiviral defense in the honey bee. *Journal of Immunology Research, 2015*.

Büchler, R., Andonov, S., Bienefeld, K., Costa, C., Hatjina, F., Kezic, N., . . . Wilde, J. (2013). Standard methods for rearing and selection of Apis mellifera queens. *Journal of Apicultural Research, 52*(1), 1-30.

Calderone, N. W. (2012). Insect pollinated crops, insect pollinators and US agriculture: trend analysis of aggregate data for the period 1992–2009. *PLOS One, 7*(5), e37235.

Camazine, S. (1986). Differential reproduction of the mite, Varroa jacobsoni (Mesostigmata: Varroidae), on Africanized and European honey bees (Hymenoptera: Apidae). *Annals of the Entomological Society of America, 79*(5), 801-803.

Cameron, R. C., Duncan, E. J., & Dearden, P. K. (2013). Stable reference genes for the measurement of transcript abundance during larval caste development in the honeybee. *Apidologie, 44*(4), 357-366.

Celle, O., Blanchard, P., Olivier, V., Schurr, F., Cougoule, N., Faucon, J.-P., & Ribière, M. (2008). Detection of Chronic bee paralysis virus (CBPV) genome and its replicative RNA form in various hosts and possible ways of spread. *Virus Research, 133*(2), 280-284.

Chen, Y., Evans, J., & Feldlaufer, M. (2006). Horizontal and vertical transmission of viruses in the honey bee, Apis mellifera. *Journal of Invertebrate Pathology, 92*(3), 152-159.

Chen, Y., Pettis, J. S., Collins, A., & Feldlaufer, M. F. (2006). Prevalence and transmission of honeybee viruses. *Applied and Environmental Microbiology, 72*(1), 606-611.

Chen, Y., Pettis, J. S., Evans, J. D., Kramer, M., & Feldlaufer, M. F. (2004). Transmission of Kashmir bee virus by the ectoparasitic mite Varroa destructor. *Apidologie, 35*(4), 441-448.

Chen, Y., Pettis, J. S., & Feldlaufer, M. F. (2005). Detection of multiple viruses in queens of the honey bee Apis mellifera L. *Journal of Invertebrate Pathology, 90*(2), 118-121.

Chen, Y. P., & Siede, R. (2007). Honey bee viruses. *Advances in Virus Research, 70*, 33-80.

Clayton, D. H., & Tompkins, D. M. (1994). Ectoparasite virulence is linked to mode of transmission. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 256*(1347), 211-217.

Cobey, S. W., Tarpy, D. R., & Woyke, J. (2013). Standard methods for instrumental insemination of Apis mellifera queens. *Journal of Apicultural Research, 52*(4), 1-18.

Cole, E. L., Empringham, J. S., Biro, C., Thompson, G. J., & Rosengaus, R. B. (2020). Relish as a Candidate Marker for Transgenerational Immune Priming in a Dampwood Termite (Blattodae: Archeotermopsidae). *Insects, 11*(3), 149.

Cremer, S., Pull, C. D., & Fürst, M. A. (2017). Social immunity: emergence and evolution of colony-level disease protection. *Annual Review of Entomology*(0).

Dainat, B., Evans, J. D., Chen, Y. P., Gauthier, L., & Neumann, P. (2012). Dead or alive: deformed wing virus and Varroa destructor reduce the life span of winter honeybees. *Applied and Environmental Microbiology, 78*(4), 981-987.

Daughenbaugh, K., Martin, M., Brutscher, L., Cavigli, I., Garcia, E., Lavin, M., & Flenniken, M. (2015). Honey bee infecting Lake Sinai viruses. *Viruses, 7*(6), 3285-3309.

de Guzman, L. I., Frake, A. M., & Simone-Finstrom, M. (2017). Comparative Flight Activities and Pathogen Load of Two Stocks of Honey Bees Reared in Gamma-Irradiated Combs. *Insects, 8*(4), 127.

de Guzman, L. I., Simone-Finstrom, M., Frake, A. M., & Tokarz, P. (2019). Comb irradiation has limited, interactive effects on colony performance or pathogens in bees, Varroa destructor and wax based on two honey bee stocks. *Insects, 10*(1), 15.

Delaplane, K. S., van der Steen, J., & Guzman-Novoa, E. (2013). Standard methods for estimating strength parameters of Apis mellifera colonies. *Journal of Apicultural Research, 52*(1), 1-12.

De Miranda, J. R., Bailey, L., Ball, B. V., Blanchard, P., Budge, G. E., Chejanovsky, N., . . . De Graaf, D. C. (2013). Standard methods for virus research in Apis mellifera. *Journal of Apicultural Research, 52*(4), 1-56.

De Miranda, J., & Fries, I. (2008). Venereal and vertical transmission of deformed wing virus in honeybees (Apis mellifera L.). *Journal of Invertebrate Pathology, 98*(2), 184-189.

De Miranda, J. R., & Genersch, E. (2010). Deformed wing virus. *Journal of Invertebrate Pathology, 103*, S48-S61.

Dietemann, V., Nazzi, F., Martin, S. J., Anderson, D. L., Locke, B., Delaplane, K. S., . . . Ziegelmann, B. (2013). Standard methods for varroa research. *Journal of Apicultural Research, 52*(1), 1-54.

Di Prisco, G., Annoscia, D., Margiotta, M., Ferrara, R., Varricchio, P., Zanni, V., . . . Pennacchio, F. (2016). A mutualistic symbiosis between a parasitic mite and a pathogenic virus undermines honey bee immunity and health. *Proceedings of the National Academy of Sciences, 113*(12), 3203-3208.

Di Prisco, G., Pennacchio, F., Caprio, E., Boncristiani Jr, H. F., Evans, J. D., & Chen, Y. (2011). Varroa destructor is an effective vector of Israeli acute paralysis virus in the honeybee, Apis mellifera. *Journal of General Virology, 92*(1), 151-155.

Di Prisco, G., Zhang, X., Pennacchio, F., Caprio, E., Li, J., Evans, J. D., . . . Chen, Y. P. (2011). Dynamics of persistent and acute deformed wing virus infections in honey bees, Apis mellifera. *Viruses, 3*(12), 2425-2441.

Doublet, V., Poeschl, Y., Gogol-Döring, A., Alaux, C., Annoscia, D., Aurori, C., . . . Bull, J. C. (2017). Unity in defence: honeybee workers exhibit conserved molecular responses to diverse pathogens. *BMC Genomics, 18*(1), 207.

Eggert, H., Kurtz, J., & Diddens-de Buhr, M. F. (2014). Different effects of paternal trans-generational immune priming on survival and immunity in step and genetic offspring. *Proceedings of the Royal Society B: Biological Sciences, 281*(1797).

Ellis, J. D., Evans, J. D., & Pettis, J. (2010). Colony losses, managed colony population decline, and Colony Collapse Disorder in the United States. *Journal of Apicultural Research, 49*(1), 134-136.

Evans, J. D. (2006). Beepath: an ordered quantitative-PCR array for exploring honey bee immunity and disease. *Journal of Invertebrate Pathology, 93*(2), 135-139.

Evans, J., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J. L., Jiang, H., . . . Hultmark, D. (2006). Immune pathways and defence mechanisms in honey bees Apis mellifera. *Insect Molecular Biology, 15*(5), 645-656.

Evans, J. D., & Spivak, M. (2010). Socialized medicine: individual and communal disease barriers in honey bees. *Journal of Invertebrate Pathology, 103*, S62-S72.

FAO. (2018). *FOASTAT*.

Ferrandon, D., Jung, A., Criqui, M. C., Lemaitre, B., Uttenweiler-Joseph, S., Michaut, L., . . . Hoffmann, J. (1998). A drosomycin–GFP reporter transgene reveals a local immune response in Drosophila that is not dependent on the Toll pathway. *The EMBO Journal, 17*(5), 1217-1227.

Fievet, J., Tentcheva, D., Gauthier, L., De Miranda, J., Cousserans, F., Colin, M. E., & Bergoin, M. (2006). Localization of deformed wing virus infection in queen and drone Apis mellifera L. *Virology Journal, 3*(1), 16.

Francis, R., & Kryger, P. (2012). Single assay detection of acute bee paralysis virus, Kashmir bee virus and Israeli acute paralysis virus. *Journal of Apicultural Science, 56*(1), 137-146.

Francis, R. M., Nielsen, S. L., & Kryger, P. (2013). Patterns of viral infection in honey bee queens. *Journal of General Virology, 94*(3), 668-676.

Freitak, D., Heckel, D. G., & Vogel, H. (2009). Dietary-dependent trans-generational immune priming in an insect herbivore. *Proceedings of the Royal Society of London B: Biological Sciences, 276*(1667), 2617-2624.

Freitak, D., Schmidtberg, H., Dickel, F., Lochnit, G., Vogel, H., & Vilcinskas, A. (2014). The maternal transfer of bacteria can mediate trans-generational immune priming in insects. *Virulence, 5*(4), 547-554.

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.

Gauthier, L., Ravallec, M., Tournaire, M., Cousserans, F., Bergoin, M., Dainat, B., & De Miranda, J. R. (2011). Viruses associated with ovarian degeneration in Apis mellifera L. queens. *PLOS One, 6*(1), e16217.

Genersch, E., Von Der Ohe, W., Kaatz, H., Schroeder, A., Otten, C., Büchler, R., . . . Gisder, S. (2010). The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. *Apidologie, 41*(3), 332-352.

Gisder, S., Aumeier, P., & Genersch, E. (2009). Deformed wing virus: replication and viral load in mites (Varroa destructor). *Journal of General Virology, 90*(2), 463-467.

Gregory, P. G., Evans, J. D., Rinderer, T., & De Guzman, L. (2005). Conditional immune-gene suppression of honeybees parasitized by Varroa mites. *Journal of Insect Science, 5*(7), 1-5.

Hamilton, C., Lejeune, B. T., & Rosengaus, R. B. (2010). Trophallaxis and prophylaxis: social immunity in the carpenter ant Camponotus pennsylvanicus. *Biology Letters, 7*(1), 89-92.

Hou, Y., Zhang, H., Miranda, L., & Lin, S. (2010). Serious overestimation in quantitative PCR by circular (supercoiled) plasmid standard: microalgal pcna as the model gene. *PLOS One, 5*(3), e9545.

Iqbal, J., & Mueller, U. (2007). Virus infection causes specific learning deficits in honeybee foragers. *Proceedings of the Royal Society B: Biological Sciences, 274*(1617), 1517-1521.

Keehnen, N. L., Rolff, J., Theopold, U., & Wheat, C. W. (2017). Insect antimicrobial defences: A brief history, recent findings, biases, and a way forward in evolutionary studies *Advances in Insect Physiology* (Vol. 52, pp. 1-33): Elsevier.

Kent, C. F., Issa, A., Bunting, A. C., & Zayed, A. (2011). Adaptive evolution of a key gene affecting queen and worker traits in the honey bee, Apis mellifera. *Molecular Ecology, 20*(24), 5226-5235.

Kevill, J. L., de Souza, F. S., Sharples, C., Oliver, R., Schroeder, D. C., & Martin, S. J. (2019). DWV-A lethal to honey bees (Apis mellifera): a colony level survey of DWV variants (A, B, and C) in England, Wales, and 32 states across the US. *Viruses, 11*(5), 426.

Khongphinitbunjong, K., de Guzman, L. I., Tarver, M. R., Rinderer, T. E., Chen, Y., & Chantawannakul, P. (2015). Differential viral levels and immune gene expression in three stocks of Apis mellifera induced by different numbers of Varroa destructor. *Journal of Insect Physiology, 72*, 28-34.

Kingsolver, M. B., Huang, Z., & Hardy, R. W. (2013). Insect antiviral innate immunity: pathways, effectors, and connections. *Journal of Molecular Biology, 425*(24), 4921-4936.

Klein, A.-M., Vaissiere, 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 of London B: Biological Sciences, 274*(1608), 303-313.

Knorr, E., Schmidtberg, H., Arslan, D., Bingsohn, L., & Vilcinskas, A. (2015). Translocation of bacteria from the gut to the eggs triggers maternal transgenerational immune priming in Tribolium castaneum. *Biology Letters, 11*(12).

Konrad, M., Vyleta, M. L., Theis, F. J., Stock, M., Tragust, S., Klatt, M., . . . Cremer, S. (2012). Social transfer of pathogenic fungus promotes active immunisation in ant colonies. *PLoS Biology, 10*(4).

Kremen, C., Williams, N. M., & Thorp, R. W. (2002). Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences, 99*(26), 16812-16816.

Kulinčević, J. M., & Rothenbuhler, W. C. (1975). Selection for resistance and susceptibility to hairless-black syndrome in the honeybee. *Journal of Invertebrate Pathology, 25*(3), 289-295.

Lanzi, G., De Miranda, J. R., Boniotti, M. B., Cameron, C. E., Lavazza, A., Capucci, L., . . . Rossi, C. (2006). Molecular and biological characterization of deformed wing virus of honeybees (Apis mellifera L.). *Journal of Virology, 80*(10), 4998-5009.

Levin, M. (1983). Value of bee pollination to US agriculture. *American Entomologist, 29*(4), 50-51.

Little, T. J., O'Connor, B., Colegrave, N., Watt, K., & Read, A. F. (2003). Maternal transfer of strain-specific immunity in an invertebrate. *Current Biology, 13*(6), 489-492.

Liu, F., Huang, W., Wu, K., Qiu, Z., Huang, Y., & Ling, E. (2017). Exploiting innate immunity for biological pest control *Advances in Insect Physiology* (Vol. 52, pp. 199-230): Elsevier.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods, 25*(4), 402-408.

Longdon, B., Cao, C., Martinez, J., & Jiggins, F. M. (2013). Previous exposure to an RNA virus does not protect against subsequent infection in Drosophila melanogaster. *PLOS One, 8*(9), e73833.

López, J. H., Schuehly, W., Crailsheim, K., & Riessberger-Gallé, U. (2014). Trans-generational immune priming in honeybees. *Proceedings of the Royal Society B, 281*(1785), 20140454.

Lourenço, A. P., Guidugli-Lazzarini, K. R., Freitas, F. C., Bitondi, M. M., & Simões, Z. L. (2013). Bacterial infection activates the immune system response and dysregulates microRNA expression in honey bees. *Insect Biochemistry and Molecular Biology, 43*(5), 474-482.

Martin, S. J., & Brettell, L. E. (2019). Deformed wing virus in honeybees and other insects. *Annual Review of Virology, 6*.

Martin, S. J., Highfield, A. C., Brettell, L., Villalobos, E. M., Budge, G. E., Powell, M., . . . Schroeder, D. C. (2012). Global honey bee viral landscape altered by a parasitic mite. *Science, 336*(6086), 1304-1306.

McMahon, D. P., Natsopoulou, M. E., Doublet, V., Fürst, M., Weging, S., Brown, M. J., . . . Paxton, R. J. (2016). Elevated virulence of an emerging viral genotype as a driver of honeybee loss. *Proceedings of the Royal Society B: Biological Sciences, 283*(1833), 20160811.

Milutinović, B., & Kurtz, J. (2016). *Immune memory in invertebrates.* Seminars in Immunology.

Möckel, N., Gisder, S., & Genersch, E. (2011). Horizontal transmission of deformed wing virus: pathological consequences in adult bees (Apis mellifera) depend on the transmission route. *Journal of General Virology, 92*(2), 370-377.

Mordecai, G. J., Brettell, L. E., Martin, S. J., Dixon, D., Jones, I. M., & Schroeder, D. C. (2016). Superinfection exclusion and the long-term survival of honey bees in Varroa-infested colonies. *The ISME Journal, 10*(5), 1182-1191.

Mordecai, G. J., Wilfert, L., Martin, S. J., Jones, I. M., & Schroeder, D. C. (2016). Diversity in a honey bee pathogen: first report of a third master variant of the Deformed wing virus quasispecies. *The ISME Journal, 10*(5), 1264-1273.

Moret, Y. (2006). 'Trans-generational immune priming': specific enhancement of the antimicrobial immune response in the mealworm beetle, Tenebrio molitor. *Proceedings of the Royal Society of London B: Biological Sciences, 273*(1592), 1399-1405.

Moret, Y., & Schmid-Hempel, P. (2001). Entomology: immune defence in bumble-bee offspring. *Nature, 414*(6863), 506.

National Research Council. (2007). *Status of pollinators in North America*: National Academies Press.

Naug, D., & Camazine, S. (2002). The role of colony organization on pathogen transmission in social insects. *Journal of Theoretical Biology, 215*(4), 427-439.

Nazzi, F., Brown, S. P., Annoscia, D., Del Piccolo, F., Di Prisco, G., Varricchio, P., . . . Pennacchio, F. (2012). Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. *PLoS Pathogens, 8*(6), e1002735.

Ongus, J. R., Peters, D., Bonmatin, J.-M., Bengsch, E., Vlak, J. M., & van Oers, M. M. (2004). Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite Varroa destructor. *Journal of General Virology, 85*(12), 3747-3755.

Peng, J., Zipperlen, P., & Kubli, E. (2005). Drosophila sex-peptide stimulates female innate immune system after mating via the Toll and Imd pathways. *Current Biology, 15*(18), 1690-1694.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution, 25*(6), 345-353.

Potts, S. G., Roberts, S. P., Dean, R., Marris, G., Brown, M. A., Jones, R., . . . Settele, J. (2010). Declines of managed honey bees and beekeepers in Europe. *Journal of Apicultural Research, 49*(1), 15-22.

Promega. (2017). *Maxwell 16 LEV simplyRNA Tissue Kit: Technical Manual*

Qiagen. (2019). QuantiTect Reverse Transcription Kit Product Details.

Rahman, M. M., Roberts, H. L., Sarjan, M., Asgari, S., & Schmidt, O. (2004). Induction and transmission of Bacillus thuringiensis tolerance in the flour moth Ephestia kuehniella. *Proceedings of the National Academy of Sciences, 101*(9), 2696-2699.

Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A., . . . Mayfield, M. M. (2008). Landscape effects on crop pollination services: are there general patterns? *Ecology Letters, 11*(5), 499-515.

Rinderer, T. E., Rothenbuhler, W. C., & Kulinčević, J. M. (1975). Responses of three genetically different stocks of the honeybee to a virus from bees with hairless-black syndrome. *Journal of Invertebrate Pathology, 25*(3), 297-300.

Rinkevich, F. D., Danka, R. G., & Healy, K. B. (2017). Influence of varroa mite (Varroa destructor) management practices on insecticide sensitivity in the honey bee (Apis mellifera). *Insects, 8*(1), 9.

Roth, O., Joop, G., Eggert, H., Hilbert, J., Daniel, J., Schmid-Hempel, P., & Kurtz, J. (2010). Paternally derived immune priming for offspring in the red flour beetle, Tribolium castaneum. *Journal of Animal Ecology, 79*(2), 403-413.

Ruttner, F. (1956). The mating of the honeybee. *Bee World, 37*(1), 3-15.

Ryabov, E. V., Childers, A. K., Chen, Y., Madella, S., Nessa, A., & Evans, J. D. (2017). Recent spread of Varroa destructor virus-1, a honey bee pathogen, in the United States. *Scientific Reports, 7*(1), 17447.

Ryabov, E. V., Childers, A. K., Lopez, D., Grubbs, K., Posada-Florez, F., Weaver, D., . . . Evans, J. D. (2019). Dynamic evolution in the key honey bee pathogen Deformed wing virus. *PLoS Biology*, 653543.

Ryabov, E. V., Wood, G. R., Fannon, J. M., Moore, J. D., Bull, J. C., Chandler, D., . . . Evans, D. J. (2014). A virulent strain of Deformed wing virus (DWV) of honeybees (Apis mellifera) prevails after Varroa destructor-mediated, or in vitro, transmission. *PLoS Pathogens, 10*(6), e1004230.

Sadd, B. M., Kleinlogel, Y., Schmid-Hempel, R., & Schmid-Hempel, P. (2005). Trans-generational immune priming in a social insect. *Biology Letters, 1*(4), 386-388.

Sadd, B. M., & Schmid-Hempel, P. (2006). Insect immunity shows specificity in protection upon secondary pathogen exposure. *Current Biology, 16*(12), 1206-1210.

Sadd, B. M., & Schmid-Hempel, P. (2007). Facultative but persistent trans-generational immunity via the mother's eggs in bumblebees. *Current Biology, 17*(24), R1046-R1047.

Salmela, H., Amdam, G. V., & Freitak, D. (2015). Transfer of immunity from mother to offspring is mediated via egg-yolk protein vitellogenin. *PLoS Pathogens, 11*(7), e1005015.

SAS Institute Inc. (2019). JMP® Pro (Version 14). Cary, NC.

Seeley, T. D., & Tarpy, D. R. (2006). Queen promiscuity lowers disease within honeybee colonies. *Proceedings of the Royal Society B: Biological Sciences, 274*(1606), 67-72.

Seitz, N., Traynor, K. S., Steinhauer, N., Rennich, K., Wilson, M. E., Ellis, J. D., . . . Caron, D. M. (2015). A national survey of managed honey bee 2014–2015 annual colony losses in the USA. *Journal of Apicultural Research, 54*(4), 292-304.

Sherman, P. W., Seeley, T. D., & Reeve, H. K. (1988). Parasites, pathogens, and polyandry in social Hymenoptera. *The American Naturalist, 131*(4), 602-610.

Škubník, K., Nováček, J., Füzik, T., Přidal, A., Paxton, R. J., & Plevka, P. (2017). Structure of deformed wing virus, a major honey bee pathogen. *Proceedings of the National Academy of Sciences, 114*(12), 3210-3215.

Slessor, K. N., Winston, M. L., & Le Conte, Y. (2005). Pheromone communication in the honeybee (Apis mellifera L.). *Journal of Chemical Ecology, 31*(11), 2731-2745.

Southwick, E. E., & Southwick Jr, L. (1992). Estimating the economic value of honey bees (Hymenoptera: Apidae) as agricultural pollinators in the United States. *Journal of Economic Entomology, 85*(3), 621-633.

Tarpy, D. R. (2003). Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. *Proceedings of the Royal Society of London B: Biological Sciences, 270*(1510), 99-103.

Tarpy, D. R., Hatch, S., & Fletcher, D. J. (2000). The influence of queen age and quality during queen replacement in honeybee colonies. *Animal Behaviour, 59*(1), 97-101.

Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M. E., & Bergoin, M. (2004). Prevalence and seasonal variations of six bee viruses in Apis mellifera L. and Varroa destructor mite populations in France. *Applied and Environmental Microbiology, 70*(12), 7185-7191.

Tidbury, H. J., Pedersen, A. B., & Boots, M. (2010). Within and transgenerational immune priming in an insect to a DNA virus. *Proceedings of the Royal Society B: Biological Sciences, 278*(1707), 871-876.

Traniello, J. F., Rosengaus, R. B., & Savoie, K. (2002). The development of immunity in a social insect: evidence for the group facilitation of disease resistance. *Proceedings of the National Academy of Sciences, 99*(10), 6838-6842.

Traynor, K. S., Rennich, K., Forsgren, E., Rose, R., Pettis, J., Kunkel, G., . . . Lopez, D. (2016). Multiyear survey targeting disease incidence in US honey bees. *Apidologie, 47*(3), 325-347.

Tzou, P., Ohresser, S., Ferrandon, D., Capovilla, M., Reichhart, J.-M., Lemaitre, B., . . . Imler, J.-L. (2000). Tissue-specific inducible expression of antimicrobial peptide genes in Drosophila surface epithelia. *Immunity, 13*(5), 737-748.

USDA-NASS. (2018). Annual Honey Report. *National Agricultural Statistics Service.*

vanEngelsdorp, D., Hayes Jr, J., Underwood, R. M., & Pettis, J. (2008). A survey of honey bee colony losses in the US, fall 2007 to spring 2008. *PLOS One, 3*(12), e4071.

Villa, J. D., & Danka, R. G. (2005). Caste, sex and strain of honey bees (Apis mellifera) affect infestation with tracheal mites (Acarapis woodi). *Experimental & Applied Acarology, 37*(3-4), 157.

Vodovar, N., & Saleh, M.-C. (2012). Of insects and viruses: the role of small RNAs in insect defence *Advances in Insect Physiology* (Vol. 42, pp. 1-36): Elsevier.

Webster, T. C., Pomper, K. W., Hunt, G., Thacker, E. M., & Jones, S. C. (2004). Nosema apis infection in worker and queen Apis mellifera. *Apidologie, 35*(1), 49-54.

Wilfert, L., Long, G., Leggett, H., Schmid-Hempel, P., Butlin, R., Martin, S., & Boots, M. (2016). Deformed wing virus is a recent global epidemic in honeybees driven by Varroa mites. *Science, 351*(6273), 594-597.

Wilson, E. O., & Hölldobler, B. (2005). Eusociality: origin and consequences. *Proceedings of the National Academy of Sciences, 102*(38), 13367-13371.

Winston, M. L. (1991). *The Biology of the Honey Bee*: Harvard University Press.

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.

Yang, X., & Cox-Foster, D. L. (2005). Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host immunosuppression and viral amplification. *Proceedings of the National Academy of Sciences of the United States of America, 102*(21), 7470-7475.

Yoo, M., Kim, I., Kang, M., Han, S., & Yoon, B. (2008). Development of real-time PCR method for black queen cell virus. *Korean Journal of Apiculture*.

Yue, C., & Genersch, E. (2005). RT-PCR analysis of Deformed wing virus in honeybees (Apis mellifera) and mites (Varroa destructor). *Journal of General Virology, 86*(12), 3419-3424.

Yue, C., Schröder, M., Bienefeld, K., & Genersch, E. (2006). Detection of viral sequences in semen of honeybees (Apis mellifera): evidence for vertical transmission of viruses through drones. *Journal of Invertebrate Pathology, 92*(2), 105-108.

Yue, C., Schröder, M., Gisder, S., & Genersch, E. (2007). Vertical-transmission routes for deformed wing virus of honeybees (Apis mellifera). *Journal of General Virology, 88*(8), 2329-2336.

Zanchi, C., Troussard, J. P., Martinaud, G., Moreau, J., & Moret, Y. (2011). Differential expression and costs between maternally and paternally derived immune priming for offspring in an insect. *Journal of Animal Ecology, 80*(6), 1174-1183.

Zheng, H.-Q., Gong, H.-R., Huang, S.-K., Sohr, A., Hu, F.-L., & Chen, Y. P. (2015). Evidence of the synergistic interaction of honey bee pathogens Nosema ceranae and deformed wing virus. *Veterinary Microbiology, 177*(1-2), 1-6.

VITA

Sarah Lang was born in Olathe, KS. She received her Bachelor of Science in Biology with an emphasis in biodiversity, ecology, and evolutionary biology from the University of Kansas in 2016. During her time at KU, she discovered her passion for entomology while working as a laboratory assistant at Monarch Watch, a nonprofit education and research program focusing on the monarch butterfly. She continued to expand her understanding of entomology working as a laboratory assistant for Dr. Kathy Denning by assisting with her PhD research on native bee communities within remnant and restored prairies in Kansas. In 2017, she began her Master of Science in entomology in Dr. Kristen Healy's lab at Louisiana State University. Her research focused on the effects of *Deformed wing virus* exposure on honey bee queens and the indirect effects on colony health and strength. She plans to continue researching and working with honey bees at the USDA Honey Bee Breeding, Genetics, and Physiology Research lab in Baton Rouge, LA.