Honeybees and their parasites

Pathogen transmission among pollinators

July 2010

Supervisors
Sjef van der Steen
Kevin Matson

Plant Research International
University of Groningen

M.P.K. Blom
1735128
Bachelor Thesis
University of Groningen
The importance of pollination is undisputed: bees perform the majority of pollination activity in natural and agro-ecosystems. However, wild and commercially reared pollinators are declining in numbers. Pathogens have been indicated as one of the main causes for these declines. Not much is known about pathogens in wild bees, whereas more information regarding commercially reared bees exists.

The European honeybee (Apis mellifera) is extensively used for pollinating monocultural crops worldwide. The use of A. mellifera in the agro-cultural industry has led to the global distribution of this pollinator. Other pollinator species such as bumble bees (Bombus spp.) have been globally distributed by humans for agricultural purposes as well. Commercially reared bees come into contact with closely related native wild bees and transmission of diseases can occur. Therefore understanding the disease ecology of honeybees can be of importance for wild bees as well.

Honeybees are susceptible for a variety of pathogens, which can be divided into 4 main groups: mites, viruses, bacteria and fungi. Honeybee pathogens can be vertically and horizontally transmitted. Vertical pathogen transmission is the transmission of pathogens from parent to offspring. Horizontal pathogen transmission is the transmission of pathogens between individuals of the same generation. Honeybee pathogens have also been transmitted between bee species. In a process called pathogen spillover, bee pathogens have been transmitted from commercially reared bees to closely related wild bee species. Pathogen spillover poses a serious threat for wild pollinator populations, however more fundamental research is necessary.

The goal of this thesis is to provide a clear overview of the most important A. mellifera pathogens and their possible modes of transmission within and among bee species. Additionally, pathogen exchange between commercially reared and wild bees will be discussed.
INTRODUCTION

The importance of pollination, provided as an ecosystem service, has been stressed many times (i.e. Costanza et al., 1997; Kearns et al., 1998; Klein et al., 2007). Plants and pollinators share a mutualistic bond in which pollinators transfer gametes between plant individuals and plants subsequently can set fruit. In return, pollinators receive nutritional resources such as pollen and nectar. Pollinators are of evident importance for natural and agro-ecosystems (Winfree, 2010). However, declines in wild pollinator populations have been recently reported (Kearns, 1997; Biesmeijer et al., 2006; Brown and Paxton, 2009; Potts et al., in press). In an extensive review, Goulson (2003) discusses the potential effects of the introduction of commercial pollinators on native ecosystems. The transmission of pathogens from non-native to native pollinators could be a cause of the decrease in wild pollinators.

Bees are the major pollinator of wild plants and crops (Winfree, 2010). Within the superfamily of bees (Apoidea), the European honeybee (Apis mellifera) is extensively used for pollinating monocultural crops worldwide. Their use in the agricultural industry is so widespread that it has led to a distribution of A. mellifera over all continents, except Antarctica (VanEngelsdorp and Meixner, 2010). Although A. mellifera is mainly used for pollination services, other pollinators are more effective for some crops. Therefore solitary bee species and bumblebees (Bombus spp.) are increasingly used for pollinating crops such as tomatoes or alfalfa (Peterson, 1992; Velthuis and Van Doorn, 2006). This process has led to global range expansions of various pollinators that are similar to the range expansion of A. mellifera. With these range expansions, commercially reared pollinators have come into close contact with wild native pollinators.

Infectious diseases in A. mellifera have been intensively studied, since disease was hypothesized as one of the main causes of population declines (Genersch, 2010c). A. mellifera can be infected by a wide range of micro- and macro- parasites and pathogens, which in turn can exert selection pressures upon honeybee populations. Infestations by parasitic mites, such as Varroa destructor, in combination with the transmission of specific viruses can induce severe colony losses (Todd et al., 2007). Much less is known about infectious diseases in wild pollinators. However, since wild and commercially reared bees can come into contact, pathogens have the potential to switch host. Therefore, knowledge about the pathogens of commercially reared bee species could be of importance for wild bee species as well.

This thesis reviews the most important A. mellifera pathogens and their transmission within and between bee species. Where appropriate, information on bumblebee pathogens is also included since bumblebees are closely related to honeybees and both are used for commercial pollination. Additionally, the potential risk of pathogen exchange between commercially reared and wild bees is discussed.
CHAPTER 1: PATHOLOGY

Honeybees are susceptible for a variety of diseases, which can be divided into 4 main groups: Mites, viruses, bacteria and fungi. The most common diseases will be discussed briefly but if present, references to more extensive studies will be given. Since this thesis focuses on the direct transmission of primary pathogens that target *A. mellifera*, indirect pathogens that target honeybee products such as honey or stored pollen, are not included in this chapter.

**Mites**

Parasitic mites are thought to play a key-role in collapsing *A. mellifera* colonies. Therefore parasitic mites are considered to be the most detrimental bee parasite in the world today (Rosenkranz et al., 2010; VanEngelsdorp and Meixner, 2010). The direct effects of the parasite itself, however, is not the main problem. Instead synergisms between parasitisation and virus infection are most harmful, since mites are capable of transmitting specific viruses. This type of interaction is discussed further in chapter two.

**Varroa destructor**

*Varroa destructor* (*varroidea*) is an obligate ectoparasitic mite that parasitizes adult honeybees but depends on bee brood for reproduction. *V. destructor* successfully shifted from its original host the Asian honeybee (*Apis cerana*) to the European honeybee. Prior to a research of Anderson and Trueman (2000), *V. destructor* was assumed to be *Varroa jacobsoni* (*varroidea*). Although *V. destructor* and *V. jacobsoni* mites are physically alike, their virulence toward *A. mellifera* is not uniform (Rosenkranz et al., 2010). *V. jacobsoni* is a parasitic mite limited to *A. cerana*: this mite can not infect *A. mellifera*. *V. destructor* is capable of colonizing both bee species (in depth description of parasitic mites of honeybees; Oldroyd and Wongsiri, 2006). The effect of *Varroa* infestations on *A. cerana* is minimal, whereas *V. destructor* infestation on *A. mellifera* can have severe consequences.

The life cycle of *V. destructor* is intimately linked to its host (Genersch, 2010b). Female mites deposit their eggs with bee larvae just before the broodcell is encapsulated. After hatching, the mites suck haemolymph through an opening in the larvae’s cuticle. This process impairs bee larvae growth (De Jong et al., 1982). The exact impact of *V. destructor* is difficult to quantify. Often colony losses can not be attributed directly to the effects of *V. destructor*. Beekeeping without *V. destructor* control, however, is nearly impossible nowadays. Without control, colonies in areas infested with *V. destructor* and a temperate climate often collapse within two to three years after contamination (Rosenkranz et al., 2010).
Viruses

At least 18 viruses have been reported to infect honeybees worldwide (Chen and Siede, 2007; Genersch, 2010b). Most of these 18 viruses may exist and even co-exist in honeybee individuals or colonies without causing any symptoms (Genersch, 2010b). However, in interplay with other pathogens and parasites such as *V. destructor*, these viruses can have an additive and lethal effect. This interplay between pathogens is discussed in-depth in chapter 2; a brief description of the most important viruses is provided here. Chen and Siede (2007) and various works by L. Baily and B.V. Ball provide more detailed reviews.

Deformed Wing Virus
Deformed Wing Virus (DWV, Iflaviridae) is one of the few bee viruses that causes well-defined disease symptoms (figure 2). Establishing causal relationships between observed symptoms and specific viruses can be a challenge in bees since many viruses are often latently present (De Miranda and Genersch, 2010). Typical symptoms of DWV infection in adult bees include shrunken crumpled wings, decreased body size and discolouration (Chen and Siede, 2007). DWV has a very high prevalence among *A. mellifera*. Recent findings in 3 studies indicate an infection rate between 90 and 100% (Tentcheva et al., 2004; Berenyi et al., 2006; Chen and Siede, 2007). However, disease is often latent and symptoms are only observed after infestation by the parasitic mite *V. destructor*. In combination with *V. destructor*, DWV can have lethal consequences. De Miranda and Genersch (2010) provide a thorough review of DWV.

Black Queen Cell Virus
After DWV, Black Queen Cell Virus (BQCV, Dicistroviridae) is the second most prevalent *A. mellifera* virus (Chen and Siede, 2007). Research on worker bees in Austrian and French apiaries detected infection rates of 30% and 86% (Tentcheva et al., 2004; Berenyi et al., 2006). As the name suggests, queen pupae are particularly susceptible. Infected workers often do not exhibit symptoms, but these workers may transmit the virus to queen pupae with the secreted brood food (Tapaszti et al., 2009). Infected queen pupae have a pale yellow appearance and a tough sac-like skin. When infection progresses, diseased pupae turn dark and die rapidly. The wall of the queen cell eventually becomes darkly colored (Chen and Siede, 2007). BQCV has a high co-incidence with the microsporidia *Nosema apis* (Tentcheva et al., 2004; Berenyi et al., 2006). Infection of the midgut by *N. apis* may increase the susceptibility for BQCV (Chen and Siede, 2007).

Acute Bee Paralysis Virus
Acute Bee Paralysis Virus (ABPV, Dicistroviridae) has been closely linked to the numerous *V. destructor* induced colony losses in Europe and the United States (Chen and Siede, 2007; De Miranda et al., 2010). ABPV is closely related to the Kashmir Bee Virus (KBV, Dicistroviridae) and Israeli Acute Paralysis Virus (IAPV, Dicistroviridae; reviewed by De Miranda et al., 2010. ABPV distribution is widespread and found on all continents except Antarctica. ABPV normally persists as a covert infection within a colony, but
the virus is extremely virulent when injected in the haemolymph (De Miranda et al., 2010). Overt infected individuals exhibit specific symptoms: trembling, paralysis and eventually death. Since virulence is high when in contact with host haemolymph, relationships between ABPV and *V. destructor* should be expected. *V. destructor* feeds on the haemolymph of hosts and likely vectors ABPV (De Miranda et al., 2010).

**Bacteria**

Prior to the arrival of *V. destructor*, two bacterial diseases affecting honeybee brood were among the most economically important diseases: European Foul Brood (EFB) and American Foul Brood (AFB). Both diseases can be lethal at the individual level and, with high infection levels, at colony level. Thereby these diseases pose a serious threat to bee health. Genersch (2010a) and Forsgren (2010) provide detailed reviews.

*European Foul Brood*

The etiological agent causing EFB is a bacterium, *Melissococcus plutonius*. This bacteria infects 4-5 day old honeybee larvae in open brood, which causes a ‘foul’ smell (Schmid-Hempel, 1998). *M. plutonius* colonizes the midgut of bee larvae after being consumed in contaminated food. Bacteria multiply within host and severe infections can have lethal consequences. A nutrient deficiency due to nutrient competition was thought to cause starvation of larvae. However, *in-vitro* experiments by McKee et al. (2004) still resulted in lethal infections even when excessive nutritional resources were provided. The exact cause of death due to EFB infection remains enigmatic (Forsgren, 2010). Brood that has died before capsulation of the brood-cells, is often removed. Adult worker bees clean out these cells and remove much of the bacteria; however, new brood can still get infected.

*American Foul Brood*

AFB (figure 1) is a major global threat to honeybee health, since it can occur frequently (5 – 10 % of colonies were infected over a 10 year observation period in Germany; Genersch, 2010b). However, in the Netherlands prevalence is very low (0 – 2 incidents per year, unpublished data). AFB is caused by the spore forming bacterium, *Paenibacillus larvae* (Genersch, 2006). Larvae are infected by consuming food with *P. larvae* spores. Vegetative bacteria colonize and massively proliferate in the midgut (Yue et al., 2008), living on food provided by the bee larvae. Eventually, the midgut is stocked with bacteria, and the epithelial barrier is breached. Proteases secreted by the bacteria may disrupt cell structure and break down cell walls.
After degrading the larvae remains to a brownish fluid, the remains desiccate and adhere to the hive cell wall as a hard scale. This scale contains numerous *P. larvae* spores that can be infectious for more than 35 years and can withstand significant changes in terms of temperature and moisture (Genersch, 2010a).

The presence of *P. larvae* can go unnoticed for some time as colonies show few, if any, symptoms (Schmid-Hempel, 1998). Individuals can contain low numbers of bacteria and develop into adults that can spread the disease in their faeces. The disease can build up latently over a period of time until symptoms appear, and the disease can subsequently lead to the collapse of a whole colony.

**Fungi**

Two fungi that can cause diseases in *A. mellifera* will be discussed; the microsporidia *Nosema spp.* and a true fungi *Crithidia mellificae*. Microsporidia have recently been added to the fungi based on their protein sequences, which are more similar to fungi than to protozoa (Cavalier-Smith, 1998; Hirt et al., 1999). Existing in-depth reviews cover *Nosema spp.* in honeybees (Chen and Huang; Fries, 2010; Higes et al., 2010). No similarly extensive review on *C. mellificae* exists to my knowledge. Two fungi (*Nosema bombi* and *Crithidia bombi*) that infect bumble bees (*Bombus spp.*) are also discussed.

**Nosema apis and Nosema ceranae**

Until the end of the previous century, *A. mellifera* was thought to be infected by only one *Nosema* species: *Nosema apis*. In 1996 another species, *Nosema ceranae*, was discovered in the Asian honeybee (Fries et al., 1996). Cross-infection of *N. ceranae* to *A. mellifera* was identified in wild specimens by Higes et al. (2006) and Huang (2007). Microsporidia are obligatory intracellular pathogens that damage many economically important insects (Schmid-Hempel, 1998). Members of this group produce spores, which are their only means to survive outside their hosts. Bees become infected by ingestion of spores (Rutrecht et al., 2007). These spores germinate by extruding a polar filament that penetrates host cells. *Nosema spp.* (figure 2) generally infect the ventricular cells of the midgut.

![Figure 2. Nosema ceranae (A) in comparison with Nosema apis (B). N. ceranae spores contain fewer polar filament (PF) coils compared to N. apis (Fries, 2010).](image)
Dysentery has been associated with infection by *N. apis*, but interestingly this agent is not the primary cause of this condition. Nevertheless, dysentery certainly aids the transmission of *N. apis* (Fries, 2010). *N. ceranae* is less associated with dysentery, which might indicate different routes of infection and transmission (Fries, 2010).

*Nosema bombi*

*Nosema bombi* is a microsporidian parasite of *Bombus spp.* (Tay et al., 2005; Larsson, 2007). *N. bombi* infects the Malpighian tubules, the ventriculus, fat tissue and nerve tissues (Fries et al., 2001). An infection by *N. bombi* can significantly reduce the lifespan of infected *Bombus spp* individuals (Otti and Schmid-Hempel, 2007). Infection occurs through the ingestion of food that contains *N. bombi* spores. Infection can be introduced into colonies via workers that were infected in the larval stage. Once this occurs, the infection is transmitted to future generations and to adults that were not previously infected (Van der Steen, 2007).

*Crithidia mellificae*

*Crithidia mellificae* belongs to the family of *Trypanosomatidae* (Langgridge and Barclay McGhee, 1967). *C. mellificae* infects the pylorum of adult honeybees but does not affect honeybees younger than 6 days. Relatively little is known about this parasite. Perhaps it cannot even be regarded as a parasite but more as a commensal since it is thought that infections cause little or no harm (Schmid-Hempel, 1998).

*Crithidia bombi*

*Crithidia bombi* (*Trypanosomatidae*) is a widespread parasite of bumblebees that can decrease colony growth and infect 10% - 30% of *Bombus spp.* (Schmid-Hempel, 1998). *C. bombi* targets the gut and infection results in large numbers of parasite cells lining the wall of the mid-gut and rectum from where cells are shed for further transmission (Schmid-Hempel, 1998). Bumblebees are annual social insects with queens that hibernate during the winter. In spring these queens found new colonies. *C. bombi* can overwinter in hibernating queens and can be transmitted in spring.
CHAPTER 2: TRANSMISSION

In this chapter I will review the modes of transmission of the most common honeybee pathogens. Additionally, I discuss modes of transmission between bee species (species host shifts).

Pathogen transmission plays an important role in the evolution of host-pathogen interactions (Schmid-Hempel, 2001). In order to maximize its own reproductive output, a parasite is dependent on its ability to transmit among hosts. If transmission is restrained, the negative effects of parasitization should be minimal due to the dependence of the parasite on its current host (Lipsitch et al., 1996). If transmission is unrestricted, the virulence of pathogens is often increased, since the pathogen is less dependent on its current host.

Pathogen transmission in eu-social bee systems can be divided into several categories (Table 1; Fries and Camazine, 2001). Vertical transmission involves the spread of disease between individuals of different generations (parent – offspring). Horizontal pathogen transmission involves the spread of disease between individuals of the same generation. Horizontal transmission can take place within a colony (intra-colonial) and among colonies (inter-colonial). Horizontal transmission can also occur between host species in a process called a host shift.

Table 1. Modes of pathogen transmission in- and between honeybee colonies (Fries and Camazine, 2001) excluding vectoral transmission.

<table>
<thead>
<tr>
<th></th>
<th>Horizontal</th>
<th>Vertical</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intracolony</strong></td>
<td>Worker to brood, worker, or drone</td>
<td>Queen to daughter (worker)</td>
</tr>
<tr>
<td></td>
<td>Drone to worker or drone</td>
<td>Queen to daughter (queen)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Queen to son (drone)</td>
</tr>
<tr>
<td><strong>Intercolony</strong></td>
<td>Worker to worker or drone</td>
<td>Swarming</td>
</tr>
<tr>
<td></td>
<td>Drone to worker or drone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(drifting, robbing)</td>
<td></td>
</tr>
</tbody>
</table>
Vertical transmission

Vertical transmission of parasites occurs with the transfer of parasites between parents and their progeny (Fine, 1975). Vertical pathogen transmission is expected to select for less virulent pathogen strains since the pathogen is dependent on the ability of its host to reproduce (Chen et al., 2006a; Fries and Camazine, 2001). If the detrimental effects of a parasite on its host are lethal, the fate of the host is shared by the parasite. Therefore vertical transmission has an important function in the long-term maintenance of viruses (Chen et al., 2006a).

Vertical transmission of pathogens in honeybees can be observed when the queen and/or drones are infected and the pathogen is transmitted to the progeny of the colony. Reproductive swarming is the main cause of inter-colonial vertical pathogen transmission. During reproductive swarming, propagules (swarms) bud off from the parent colony to start new colonies (Fries and Camazine, 2001). Swarms are often headed by the old (infected) queen, which leaves the old colony behind with a new queen (or queen larva) and a part of the (infected) worker force. In this process of reproductive swarming, two infected colonies can arise from a single infected colony.

Viruses

Viruses in honeybees can be transmitted from parent to offspring. Chen et al. (2005) checked honeybee queens for the prevalence of six viruses and found five viruses (including DWV, BQCV and ABPV) in these samples. Virus prevalence in queens can be an indication of the vertical transmission of viruses from mother to offspring since queens are responsible for the whole progeny of a colony. Chen et al. (2006b) observed a correlation between viruses present in queens and viruses present in the offspring of queens (eggs, larvae and adult workers). Honeybee queens that didn’t harbor viruses, produced non-infected progeny. A similar result was obtained by De Miranda and Fries (2008), who infected virgin queens with DWV particles and observed progeny with an almost 100% infection rate. These viruses are able to infect the ovary tissue of queen bees. However, viruses are likely in a latent stage since a deleterious effect on embryos was not observed (Chen et al., 2006a).

Besides the ovaries of honeybee queens, the semen of drones has also shown to harbor viruses. A study by Yue et al. (2006a) on the semen of honeybee drones has shown that bee sperm can contain multiple viruses, indicating a venereal transmission pathway. Venereal transmission occurs, when diseases are transmitted to the opposite sex or progeny, through mating. Yue et al. (2006b) experimentally fertilized DWV-negative unfertilized eggs with DWV-positive sperm and they observed that all of their fertilized honeybee eggs were DWV infected. Interestingly, under natural conditions the relative frequency of viral infections of semen did not correspond with the relative frequency of the virus in the bee population (Yue et al., 2006a). For example DWV is present in almost 100% of the populations, however often in latent state, whereas only 50% of the semen samples was infected by DWV.
**Fungi**

In contrary to other insect species (Van Frankenhuyzen et al., 2007; Goertz and Hoch, 2008), there is no evidence for the vertical transmission of fungi in honeybees. Webster et al. (2008) infected honeybee queens with *N. apis* and checked queens for disease development. Six out of seven queens developed a *N. apis* infection in their ventriculi, but none had infected ovaries and neither was their progeny infected. In contrary, a similar study on the closely related microsporidia *N. bombi* found infection of the ovaries of *Bombus lucorum* individuals (Rutrecht and Brown, 2008). Moreover, *C. bombi*, a parasite of bumblebees (*Bombus spp.*) can be transmitted from mother to offspring. Bumblebee queens hibernate during winter and found new colonies in spring. *C. bombi* is able to hibernate during winter in these queens and subsequently infect the spring progeny (Youth et al., 2008).

**Bacteria**

When swarming honeybee individuals move to new colonies, AFB spores can be transmitted to the new colony. A study by Fries et al. (2006) indicated that swarms can carry spores of *P. larvae* and these spores can cause infections in daughter colonies. Interestingly, in addition to the colonies that were latently infected, three colonies were collected that swarmed even though these colonies exhibited clinical symptoms of AFB. Swarming by diseased colonies can have stronger implications for vertical transmission than swarming of colonies by latently infected AFB colonies, since pathogen load is higher in diseased colonies. Infected colonies that swarmed reduced the number of *P. larvae* spores present in infected individuals. The high spore load levels that are required to create diseased colonies of AFB, are therefore not easily transmitted (Fries et al, 2006). Swarming can perhaps be used to reduce bacterial pathogen load. Dutch beekeepers create swarms in order to reduce the pathogen load of their honeybee colonies (personal communication).

**Horizontal transmission**

Horizontal pathogen transmission is the transmission of pathogens between individuals of the same generation (Chen et al. 2006a) both intra- and inter colonial. Intra-colonial horizontal transmission is of importance in eu-social bee systems where population density in hives is high and frequent physical contact between individuals is common. Physical contact is promoted through grooming practices and intimate feeding activities involving regurgitation (trophallaxis).

Inter-colonial horizontal transmission is also important in eu-social bee systems and it can occur in various ways (Fries and Camazine, 2001). Drifting occurs when bees accidentally enter colonies other than their own (Pfeiffer and Crailsheim, 1998; Birmingham et al., 2004). Drifting often occurs when multiple colonies are concentrated in a small area. Wild honeybee colonies are scattered over large distances, but commercialized honeybee colonies are often more densely concentrated in a manageable area.
Occasionally honeybees enter hives other than their own on purpose, in a process called robbing. When foraging resources are limited, bees enter weaker hives and rob their nutritional resources. Healthy honeybee colonies guard their hives and repel robbing bees; colonies that are weakened by diseases are less able to fend off intruders. Therefore, honeybees that rob are exposed to the same pathogens responsible for weakening the invaded colony (Fries and Camazine, 2001).

Individuals can come into contact with individuals from other colonies while outside of their hive, and pathogens can be transmitted. Individual flowers are visited multiple times by different pollinators. Shared use of flowers can lead to the horizontal transmission of a bumblebee pathogen, *C. bombi* (Durrer and Schmid-Hempel, 1994). Other pathogens might also be transmitted through the shared use of flowers, and flowers therefore might represent hotspots for the horizontal transmission of bee pathogens.

Since flowers are the central basis of all pollination activity, pathogen transmission through the shared use of flowers can have severe implications on pollinator ecology and evolution. However up to now only a single, much quoted, article (Durrer and Schmid-Hempel, 1994) exists upon this matter. Therefore further research is necessary to determine the likelihood of flowers serving as vectors in the transmission of pathogens. Perhaps flowers are a vital link in the transmission of pollinator pathogens.

**Mites**

*V. destructor* can be horizontally transmitted within and between honeybee colonies. The mite is closely linked to its *A. mellifera* host and lacks a free-living stage (Figure 3; Rosenkranz et al., 2010). The males and nymphal stages of the mite do not leave the brood cells. Adult female mites are able to infest adult worker bees and the horizontal transmission of mites within and between honeybee colonies takes place with the dispersal of worker bees. Mites can be dispersed between colonies through swarming, foraging, or other activities when adult bees from different colonies interact (Kuenen and Calderone, 1997).

![Fig. 3. Opened brood cells with Varroa destructor infested A. mellifera larvae (Rosenkranz et al., 2010)](image-url)
Viruses

Viruses are horizontally transmitted between individuals via different pathways. Nutritionally related activities such as foraging, are among the main activities of honeybees. Bees gather nectar, pollen and water from their environment and return it to the colony. Upon return in the colony, worker bees regurgitate food and pass it on to nursery bees that add saliva and store the food product in the colony. In times of need, nutritional resources that have been stored are used to feed the colony. Worker honeybees feed on stored resources and feed other colony individuals by regurgitating the food. Viruses are spread among individuals via these foraging and processing pathways.

The foodborne transmission pathway is an excellent carrier for pathogens to jump host. Shen et al. (2005a) and Chen et al. (2006a) detected viruses in colony food, including honey, pollen and royal jelly. The presence of viruses in colony food suggests that colony food can be involved in the spread of virus infections (Chen and Siede, 2007). The elevated presence of virus particles in gut tissue of honeybee queens, in comparison with other body tissues, is another indication for a food-borne transmission pathway (Chen et al., 2006b). However, it is unclear how many virus particles must be ingested to lead to an infection. Other pathways of horizontal virus transmission include venereal transmission and air-borne transmission of virus particles. Chen and Siede (2007) review horizontal virus transmission in a chapter on honeybee pathology.

The importance of vector-borne transmission of viruses has been stressed by recent research (Chen et al., 2004; Shen et al, 2005a; Shen et al, 2005b; Chen et al., 2006; Chen and Siede, 2007; Todd et al., 2007; Dainat et al., 2009). Vector-borne transmission is an indirect route of horizontal transmission. This process involves an intermediate biological host, a vector, which acquires and transmits viruses from one host to another (Chen and Siede, 2007). The most prominent vector of honeybee viruses is *V. destructor*. These mites are capable of transmitting viruses between hosts in the process of feeding on host haemolymph. Bowen-walker et al. (1999) first suggested the spread of DWV by *V. jacobsoni* (probably *V. destructor*) and subsequent studies have confirmed the spread of DWV by *V. destructor* (Tentcheva et al., 2004; Shen et al., 2005). Since this discovery, multiple other viruses (incl. BQCV) have been found able to be transmitted by this mite (Chen et al., 2004; Shen et al., 2005; Chen and Siede, 2007; Todd et al., 2007).

Besides transferring viruses between hosts, *V. destructor* also functions as an activator of latent viruses that are already present in the host prior to *V. destructor* infestation (Shen et al., 2005). Additionally, viruses have also been found capable to replicate themselves within *V. destructor* and to be transmitted between *V. destructor* individuals (Ongus et al., 2004). *V. destructor* is therefore a very potent and harmful biological vector to transmit viruses between *A. mellifera* individuals.
**Bacteria**

AFB is a frequently occurring and a potentially deadly disease of honeybees (Genersch, 2010b), but not much is known of its horizontal transmission. A study by Lindstrom et al. (2008) indicates that horizontal transmission of AFB may occur when honeybees from healthy colonies invade and rob diseased colonies. The authors stress the importance of distance between colonies, since neighbouring colonies have a higher likelihood to be robbed than distant colonies. Additionally, colonies contained adults with high spore loads without causing clinical disease symptoms. It is unclear why these high spore loads do not lead to AFB infection with clinical symptoms. The functional mechanism behind transmission and the actual establishment of the disease in its host should be further explored. A similar result was observed when the etiological agent causing European Foulbrood was tested for horizontal disease transmission (Roetschi et al., 2008). Adult worker honeybees carried high spore loads of *M. plutonius* but the high spore load did not correlate with the prevalence of disease development in larvae.

**Fungi**

Horizontal transmission of fungi between colonies can occur through drifting, shared use of flowers and robbing. Rutrecht et al. (2007) observed the horizontal transmission of the microsporidia *N. bombi* in bumblebee species. Interestingly, the virulence of *N. bombi* among individuals is low, which is unexpected since horizontal transmission tends to select for a high virulence of pathogens in comparison to vertical transmission. Horizontal transmission ought to select for high levels of virulence since the parasite is less dependent on the direct reproduction success of its current host, in contrary to the vertical transmission of parasites (Fries, 2001; Chen et al., 2006a). The low level of *N. bombi* virulence among individuals, presumably is an indication that the parasite-host relationship has reached equilibrium. Co-evolution has led to stability as a result of a long-term parasite-host relationship (Royce and Rossignol, 1990).

The success of horizontal transmission also depends on social interaction. Otterstatter and Thomson (2007) observed the social interactions of bumblebees (*Bombus impatiens*) from colonies infected with *C. bombi*. Individuals that interacted more frequently with other individuals of the colony, were more likely to be infected with *C. bombi*. Additionally, differences in rates of infection among colonies resulted largely from differences in network density among hives.
Inter-species host shifts

Besides shifting host within a single species of bees, some pathogens are able to shift between species of bees as well. Inter-species host shifts are pathogen shifts between hosts of different species. Of particular interest is the shifting of pathogens between commercially reared and wild species. Pathogen spillover occurs when epidemics in a host population are not driven by pathogen transmission within that population, but by the transmission of pathogens from a closely related reservoir population (Power and Mitchell, 2004). Due to the global distribution of various commercialized bee species, concerns regarding pathogen spillover in bees exist (Colla et al., 2006; Goulson et al., 2008; Otterstatter and Thomson, 2008). Commercialized pollinators are kept in much higher densities by beekeepers, than the densities that naturally occur in the wild. The rate of pathogen transmission, either within domestic populations or between domestic and wild populations, is increased by high host densities (Power and Mitchell, 2004). Commercially reared bee species can function as parasite reservoirs, which can lead to the spillover of pathogens to wild bee species.

Colla et al. (2006) compared the prevalence of pathogens in wild bumblebees caught in the vicinity of commercial greenhouses with the prevalence of pathogens in wild bumblebees caught in areas distant from commercial greenhouses (fig. 4). Commercially reared bumblebees were used for the pollination of crops in these greenhouses.

![Fig. 4. The infection rate of 4 diseases in wild bumblebees. Wild bumble bees collected in the vicinity of commercial greenhouses have a higher pathogen load than wild bumblebees collected at a site distant from commercial greenhouses. Greenhouses use commercial bumblebees for the pollination of their crops (Colla et al., 2006)]
The results of Colla et al. (2006) indicated that wild bumblebees caught in the vicinity of greenhouses have a higher pathogen load than wild bumblebees caught in areas distant from greenhouses. A similar result was obtained by Otterstatter and Thomson (2008), who found a decrease in C. bombi and N. bombi prevalence in wild bumblebee species, with increasing geographical distance from greenhouses where commercially reared bumblebees were used for pollination activities.

Commercially reared bee species interact with wild pollinators when they escape their designated area of pollination. A majority (73%) of the pollen collected by bumblebees in a tomato greenhouse, originated from plants outside the greenhouse in a study by Whittington et al. (2004). Commercially reared pollinators therefore can come into contact with closely related wild species. Durrer and Schmid-Hempel (1994) showed how the shared use of flowers could lead to the horizontal transmission of Crithidia bombi between bumblebees. Interestingly, C. bombi is also increasingly prevalent in wild bumblebee species in vicinity of greenhouses with commercially reared bumblebees (Colla et al., 2006; Otterstatter and Thomson, 2008). Therefore it is likely that C. bombi is transmitted from commercially reared bee species to wild bee species at wild flowers in the vicinity of greenhouses.

Since V. destructor has jumped host from A. cerana to A. mellifera, it has been one of the most harmful parasites of the European Honeybee. V. destructor has not such detrimental effects on A. cerana, as it has on A. mellifera. The long existing parasite-host relationship observed in A. cerana, has presumably coevolved to a stable equilibrium (Royce and Rossignol, 1990; Oldroyd, 1999), whereas the recent introduction of V. destructor in A. mellifera has possibly initiated an arm’s race between parasite and host. An example of adaptation of A. cerana to the presence of V. destructor, is the grooming of workerbees that have been outside of the colony. Due to grooming, mites are removed from the bee fur. Such adaptations have not yet evolved in A. mellifera.

Two strains of V. destructor on A. mellifera have been discovered (Oldroyd, 1999; Rosenkranz et al., 2010), indicating that the host shift from A. cerana to A. mellifera has occurred two times independently from one another. There is not much clear, when and how this shift has taken place. General assumption is that with the transport of A. mellifera individuals to the far east, somewhere in the first half of the past century, V. destructor was given the possibility to move to a new host (Oldroyd, 1999). Since the host shift, infested A. mellifera colonies have been moved around the world and the V. destructor infestation of A. mellifera has spread.
*N. ceranae* is a microsporidian parasite that is able to infect multiple honeybee species. *N. ceranae* was first described as a parasite of *A. cerana* (Fries et al., 1996) but has shown to be cross infective with *A. mellifera* (Higes et al., 2006). When and where this host shift has taken place is unclear, but the oldest *A. mellifera* sample known to be infected with *N. ceranae* is a 20 year old sample from Uruguay (Invernizzia et al., 2009). Interestingly, *N. ceranae* seems to be replacing *N. apis* in *A. mellifera* over the last couple of years. This replacement remains poorly understood since the spores of *N. ceranae* are less durable than the spores of *N. apis* (Fries, 2010). *N. ceranae* was thought to be host-specific to honeybees, but Plischuk et al. (2009) reported the infection of native South-American bumblebees. *N. ceranae* was detected in two geographically distant populations, implying a wide dispersion of this pathogen. In this region of South-America, *N. ceranae* has been linked to significant honeybee colony losses. The high prevalence of *N. ceranae* in this area, has possibly led to a *N. ceranae* host shift from *A. mellifera* to native South-American bumblebee species.

Not much is known about viruses shifting among host bee species. European honeybee viruses such as BQCV and DWV are known to infect Asian honeybees as well (Oldroyd and Wongsiri, 2006). Genersch et al. (2006) reported on two natural cases in which a virus of honeybees, DWV, was observed in wild bumblebee species (figure 5). Which might indicate a host shift and a broader host range for this previously assumed honeybee host-specific pathogen.

*Fig. 5. Bombus terrestris individual with DWV disease (left) and a healthy Bombus terrestris individual (right; Genersch et al., 2006)*
CONCLUSION

The importance of pollination, by commercially reared or wild pollinators, is undisputed. However, recent reports suggest that wild pollinator numbers are declining (Biesmeijer et al., 2006; Potts et al., in press). Commercially reared pollinators such as *A. mellifera* and *Bombus terrestris*, have been studied extensively, and some of the diseases affecting them are well-understood (Genersch, 2010b). Disease might be one of the primary causes of honeybee colony collapses (Genersch, 2010c). This thesis summarized bee pathogens and their routes of transmission. Additionally, I summarized the existing knowledge about pathogen transmission between bee species in order to provide a background for further studies on the pathogen spillover from commercially reared bees to wild bees.

Bee pathogens can be transmitted through various routes. Due to the eusocial system of honeybees, individuals have close contact with one another in colonies with high population densities. These circumstances are ideal for pathogen transmission between hosts within a colony. Transmission between colonies occurs through processes such as swarming, drifting and robbing. Additionally, research has shown that the spores of various fungi can be transmitted between bees through the shared use of flowers. More research should be dedicated to elucidate the likelihood of flowers serving as pathogen vectors, since flowers fulfil a central role in pollinator ecology.

Bee pathogens have shown to be very versatile in adapting new ways to infect hosts. Viruses have infected ecto-parasitic mites and are able to be transmitted between bee hosts through mite infestations. The mite *V. destructor* has infested *A. mellifera*, after this species was introduced in its native range where the mite used to parasitize *A. cerana*. Since this initial host shift, *V. destructor* has rapidly infested honeybees worldwide and pose a serious threat to colonies, especially in synergism with viruses.

Due to the adaptive capabilities of bee pathogens, globally distributing commercially reared bees should be considered with care. The spillover of pathogens from commercially reared bee species to closely related wild species, could have severe consequences. Recent infection discoveries of *N. ceranae* and DWV in wild bumblebee species (Genersch et al., 2006; Plischuk et al., 2009) fuel the necessity to conduct further research, since these pathogens were thought to be host specific to honeybees. The results of such studies should not be taken lightly. If commercially reared bees are indeed a reservoir of pathogens that are capable to be transmitted to wild species, than the use and distribution of commercially reared pollinators should be evaluated. Given the importance of wild pollinators for the pollination in our ecosystems.
REFERENCES


Colla, S.R., Otterstatter, M.C., Gegear, R.J. and Thomson, J.D. 2006. Plight of the bumblebee: Pathogen spillover from commercial to wild populations. *Biological conservation* **129**: 461-467


Oldroyd, B.P. and Wongsiri, S. 2006. Asian honeybees: Biology, Conservation and Human Interactions. *Harvard University Press*


Shen, M.Q., Cui, L.W., Ostiguy, N. and Cox-Foster, D. 2005a. Intricate transmission routes and interactions between picorna-like viruses (Kashmir
bee virus and Sacbrood virus) with the honeybee host and the parasitic varroa mite. *J. Gen. Virol.* **86**: 2281-2289


VanEngelsdorp, D. and Meixner, M.D. 2010. A historical review of managed Honeybee populations in Europe and the United States and the factors that might affect them. *Journal of Invertebrate Pathology* **103**: S80-S95


