Factors impacting honeybee colony vitality: from the individual to the population level

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General introduction

Honeybees (*Apis mellifera* L.) are eusocial insects belonging to the order of Hymenoptera. They are often referred to as cornerstone or key pollinator insects due to their tremendous pollination ability. As generalist pollinators, wild and managed honeybees contribute not only to the maintenance of wild plant communities but also to the productivity of most agricultural crops (Williams 1994; Roubik 1995; Klein *et al.* 2007). Indeed, it has been estimated that 1330 tropical and 264 European crop species depend directly or indirectly on animal pollination (Williams 1994; Roubik 1995). Klein *et al.* (2007) reported that up to 35% of global crop production depends on pollinating animals, with more than 90% attributed to honeybees themselves. Consequently, the pollination provided by honeybees is of colossal economic value, which has been estimated to exceed €153 billion worldwide (Gallai *et al.* 2009). In addition to this pecuniary aspect of honeybee services, honeybees are thought to pollinate 80% of wild flowers (De la Rúa *et al.* 2009). Since the demand for pollination is overcoming the global stock of honeybees (Aizen & Harder 2009), their conservation and management is crucial for the maintenance of biodiversity, the global food web and human health.

Honeybees exhibit extremely specialized and complex haplodiploid mating systems and exceptionally high levels of social organization (Wilson 1971). As eusocial insects, honeybees live in close cooperation in large and dense colonies resembling superorganisms (Moritz & Southwick 1992; Moritz & Fuchs 1998). A honeybee colony is structured in three specialized individuals: a sole one undertaking reproduction, the queen; the few hundred ones mating the queen and only present during spring, the males (drones); and the most numerous (from 15000 to 50000, depending on the season) non-reproductive altruistic ones, the workers. Typically, worker bees perform all multiple and diverse tasks, including cleaning cells, rearing brood, tending the queen, guarding and foraging (Wilson 1971).

Honeybee queens are exceptionally highly polyandrious (*i.e.* they mate with many males; Moritz & Fuchs 1998) and more than 10 drones simultaneously father offspring within a single colony (Neumann & Moritz 2000). Mating occurs on the wing at Drone Congregation Areas (=DCAs, Zmarlicki & Morse 1963), where thousands of drones from the surrounding colonies aggregate (Koeniger *et al.* 2005). After mating, the queen lays unfertilised and fertilised eggs, from which males and female workers arise respectively, with all following four developmental life stages: egg, larva, pupa and adult (Wilson 1971). Being haploid individuals, males only have one half of the genetic material carried by the queen, while daughters from the same patrilines share the same paternal genotype, and thus, are highly genetically related. This

intracolonial worker-worker relatedness has been suggested to be a key component for the evolution of eusociality and altruistic behaviour (Hamilton 1964). Therefore, the evolution of extreme polyandry in the honeybee queen runs counter to Hamilton's kin selection theory because polyandry reduces intracolonial relatedness (Nowak *et al.* 2010). Several hypotheses have been formulated to explain how the benefits of intracolonial genetic diversity generated through multiple matings outweigh the costs of reduced altruism resulting from low within-colony relatedness (*e.g.* Page 1980; Crozier & Page 1985; Palmer & Oldroyd 2000, 2003; Crozier & Fjerdingstad 2001), but no universally applicable conclusion has yet been reached (Strassmann 2001). Colonies with multiple patrilines show enhanced colony efficiency, with a more resilient system of division of labour improving their adaptation and response to environmental changes (Fuchs & Moritz 1999; Tarpy 2003; Jones *et al.* 2004; Mattila & Seeley 2007, 2014; Oldroyd & Fewell 2007; Delaplane *et al.* 2015). Tarpy (2003) demonstrated that polyandry reduces the variance of important parameters that contribute to colony fitness (*i.e.* brood viability, hygienic behaviour) and the variance of disease. Furthermore, Seeley & Tarpy (2007) showed that colonies with higher genetic diversity had lower disease intensity and higher colony strength. Irrespective of why honeybees evolved extreme polyandry, the ability of a queen to mate with multiple drones is paramount to the fitness of a colony.

Honeybees have evolved sophisticated orientation and communication systems to regulate the colony and function as an adaptive unit. Typically, honeybees execute several orientation flights during their early life time to locate their colony using visual, auditory, and magnetic cues (Wilson 1971). Once they learn how to locate their own colony, they can fly away either to mating sites, in the case of the queen and drones, or to foraging sites, in the case of the worker bees, and return to their respective colony. Orientation mistakes of returning bees, also known as drifters, result in the bee entering the wrong colony (Free 1958). This behaviour may have consequences for both the source and the host colonies of the drifter, for instance, drifting bees show lower levels of activity compared to nestmate workers (Pfeiffer & Crailsheim 1999). Furthermore, drifting is also a major pathway for transmission of intracolonial disease as drifters can introduce new pathogens in the host colony or carry back pathogens contracted in the host colony to their home colony (Fries & Camazine 2001). Therefore, orientation is a paramount feature of colony for reproduction, food foraging and health.

Communication is also fundamental for colony functioning and regulation. Honeybees possess an extraordinary rich chemical language of pheromones which are spread among all the

nestmates through a complex web of social interactions, such as antennation and trophallaxis (Wilson 1971; Slessor *et al.* 2005). This elaborate system of communication is a key factor in generating and maintaining the complexity of honeybees' society by allowing coordinated division of labour, regulation of colony homeostasis and social cohesiveness (Slessor *et al.* 2005; Trhlin & Rajchard 2011). Communication is important for almost every aspect of the colony function, including mating, swarming, defence, orientation, recognition and cohesiveness (Bortolotti & Costa 2014).

The defensive response is a typical example of collective behaviour based on recruitment and amplification processes (Millor *et al.* 1999). Guarding bees patrol the hive entrance by inspecting all individuals entering through antennation to discriminate between nestmates and non-nestmates (*e.g.* drifting bees; Moore *et al.* 1987). In the case of actual or potential danger, they release alarm pheromones to inform of the threat and recruit other nestmates to elicit an adapted collective defensive response. Honeybee defence behaviour illustrates self-organisation that coordinates every colony function. Superorganism-like functioning of the colony implies that decisions are made based on local stimuli (Moritz & Fuchs 1998), with individual bees responding to local behavioural interactions with nestmates or their local environment to modulate a specific task (Seeley *et al.* 1991; Seeley 1995; Watmough & Camazine 1995). Honeybees have evolved efficient decentralised control of colonial decision-making based on the integrity and functioning of local social networks (Seeley *et al.* 1991). Self-organisation as a mechanism through local information exchange eliminates the need for a time-consuming communication between the peripheral sensor/effector individuals and the actual central decision-making. The self-organised structures shape the colonial phenotype and are affected by natural selection (Moritz & Fuchs 1998). Since orientation and communication are involved in the performance of almost all colony tasks, they have profound influence on colony vitality.

Colony vitality refers to the adaptability of a colony to a given environment at a given time and comprises both colony health and colony fitness. The process of adaptation can happen naturally by natural selection, but in the case of managed colonies, the beekeeper may have a strong influence (Meixner *et al.* 2010). Thus, both endogenous (*i.e.* genetic variability of the colony) and exogenous factors, such as the environment (including pesticides), pests and pathogens as well as beekeeping practices have profound impact on colony vitality (Costa *et al.* 2012). Several parameters have been proposed to assess colony vitality such as adult and brood quantity, honey and pollen quality and quantity, swarming tendency, and hygienic behaviour (Costa *et al.* 2012). Nowadays, there is an increasing interest in understanding the factors

affecting vitality as well as their interactions for the comprehension of the current threats altering colony vitality.

A drastic decrease in the number of managed honeybee colonies has been reported in the USA (Ellis *et al.* 2010; vanEngelsdorp *et al.* 2010) and in Europe (Potts *et al.* 2010a) over recent years. Furthermore, wild and feral honeybee colonies are also declining on a global scale (Potts *et al.* 2010a). Research has suggested many drivers contributing to those declines, including intensive land use with the consequent loss and fragmentation of suitable nesting and foraging habitats, climate change, introduction of alien species, pesticide pollution, spread of foreign pest, pathogens and parasites (*e.g.* Neumann & Carreck 2010; vanEngelsdorp & Meixner 2010; Williams *et al.* 2010; Potts *et al.* 2010b; Le Conte *et al.* 2012; Martin *et al.* 2012; González-Varo *et al.* 2013; Goulson *et al.* 2015). Detrimental beekeeping practices and loss of incentives for beekeeping may also contribute to the loss of honeybee colonies (De la Rúa *et al.* 2009). Despite extensive comprehensive research efforts (*e.g.* Cox-Foster *et al.* 2007; Stokstad 2007), specific factors causing the ill-famed "colony collapse disorder" (CCD) responsible for the death of millions of colonies in the USA in 2006 (vanEngelsdorp *et al.* 2009) and the elevated overwintering mortality are still poorly understood.

Today, it is broadly accepted that numerous potential interactions between multiple factors, especially between pests, pathogens and pesticides, and seasonal and regional differences between their multiple effects are involved in those losses (Bailey & Ball 1991; De la Rúa *et al.* 2009; Neumann & Carreck 2010; Potts *et al.* 2010a; Nguyen *et al.* 2010; vanEngelsdorp & Meixner 2010; Dainat *et al.* 2012; McMenamin & Genersch 2015). For example, studies provided evidence that the microsporidia *Nosema*, in combination with pesticides, increased the susceptibility of honeybees to fungal infection (Alaux *et al.* 2010; Vidau *et al.* 2011; Aufauvre *et al.* 2012; Wu *et al.* 2012; Doublet *et al.* 2015). Both wild and managed honeybee colonies may be differentially affected by these pathogens. Indeed, the recent worldwide spread of the ectoparasitic mite *Varroa destructor* has resulted in the dramatic loss of wild and feral colonies over the past decades, leaving only the managed colonies most likely due to protective measures taken by beekeepers (Kraus & Page 1995; Rosenkranz *et al.* 2010).

The ectoparasitic mite *V. destructor* (formerly also *V. jacobsoni*; Anderson & Trueman 2000) emerged as a novel parasite of *A. mellifera* after a switch from its natural host, the Asian honeybee *Apis cerana* (Rosenkranz *et al.* 2010). The new host-parasite co-evolution relationship did not allow *A. mellifera* to develop resistance against the *Varroa* mite and infested colonies show higher wintering mortality without treatment (Rosenkranz *et al.* 2010;

Frey & Rosenkranz 2014). Honeybee importations have facilitated the quick spread of *V. destructor* to almost all parts of the world (Oldroyd 1999; Boecking & Genersch 2008), with only Australia (Rosenkranz *et al.* 2010) and extremely isolated populations remaining *Varroa* free (*e.g.* Tentcheva *et al.* 2004).

The *Varroa* life cycle is composed of two different steps, the phoretic phase and the reproductive phase (Rosenkranz *et al.* 2010). During the phoretic phase, the mite on an adult bee feeds on the hemolymph and infested adult bee serves as a physical vector for the mite to be transmitted within and between colonies. To complete its life cycle, the mite needs to invade honeybee larval cells to feed on the developing pupae and reproduce. By feeding on the hemolymph, *Varroa* causes a variety of damage at both individual and colony levels. For instance, infested foraging workers display a decreased capability of non-associative learning and their homing success (Kralj & Fuchs 2006; Kralj *et al.* 2007), potentially due to a reduced ability to navigate (Ruano *et al.* 1991). *Varroa* infestation affects colony reproduction in two ways: 1) parasitized drones have decreased flight performance, and therefore a lower chance to mate (Duay *et al.* 2002), and 2) infested colonies have a reduced swarming capacity (Fries *et al.* 2003; Villa *et al.* 2008). Therefore, the *Varroa* mite has been suggested to play a central role in colony losses, since regions with established *Varroa* mite populations suffered from greater colony losses than regions without (Dahle 2010). The mite is now considered the greatest menace to honeybee health and beekeeping (Boecking & Genersch 2008; Rosenkranz *et al.* 2010). Nevertheless, since most managed honeybee colonies are infested by *Varroa*, it is unlikely that the mite can be the only cause of all the recent losses (Neumann & Carreck 2010). Since the introduction of the mite, wild and feral honeybee populations in Europe and North America have been nearly completely eradicated, thus, sustainable *Varroa* control methods are essential for keeping managed honeybee colonies alive.

In addition to the numerous deleterious and direct effects caused by *Varroa* infestation, the mite also serves as a physical and biological vector in transmitting viruses thereby adding to the pathology of mite feeding injuries (Ball & Allen 1988; Bailey & Ball 1991; Bowen-Walker *et al.* 1999; Martin 2001; Chen *et al.* 2004; Shen *et al.* 2005; Yang & Cox-Foster 2007; Martin *et al.* 2012). The close association between viruses and their vectors may have substantially contributed to their worldwide spread (Genersch & Aubert 2010). Most honeybee-infecting viruses are positive-stranded RNA viruses, with many of them associated with varying degrees to *Varroa* (*e.g.* Deformed wing virus (DWV), Acute bee paralysis virus (ABPV), Israeli acute bee paralysis virus (IAPV), Black queen cell virus (BQCV), Slow bee paralysis virus (SBPV), Sacbrood virus (SBV); Ball 1983; Ball & Allen 1988; Allen & Ball 1996; Martin 2001,

2012; Tentcheva *et al.* 2004; Chen & Siede 2007; Boecking & Genersch 2008; Carreck *et al.* 2010; Genersch & Aubert 2010; Di Prisco *et al.* 2011). Only three of them, alone or in co-infection, have been associated to both CCD in the United States and winter colony losses in Europe (*i.e.* DWV, ABPV and IAPV; Cox-Foster *et al.* 2007; Highfield *et al.* 2009; Berthoud *et al.* 2010; Genersch *et al.* 2010; Dainat *et al.* 2012; McMenamin & Genersch 2015). The co-infestation of *Varroa* and their vectored viruses may have a negative impact on colony fitness by affecting the immune response of honeybees making them more susceptible to disease (Gregory *et al.* 2005; Yang & Cox-Foster 2005, 2007).

It is worth mentioning that many honeybee-infecting viruses infect other bee species (Singh *et al.* 2010; Levitt *et al.* 2013; Parmentier *et al.* 2016). Inter-species transmission of these viruses can occur in natural settings via shared floral resources (Singh *et al.* 2010; McMahon *et al.* 2015). Recently, it was demonstrated that sympatric honeybee and bumblebee populations share similar virus strains (Fürst *et al.* 2014; McMahon *et al.* 2015).

The *Varroa* mite and its associated viruses are not the only pathogens that both beekeepers and bee scientists must face nowadays. The microsporidia *Nosema* spp. is now also almost as globally widespread as the mite (Klee *et al.* 2007; Paxton *et al.* 2007). *Nosema* is an obligate intracellular parasite, horizontally transmitted via spore ingestion, most likely through the activities of cleaning and trophallaxis, which infects the honeybee midgut causing nutritional and energetic stress (Higes *et al.* 2006, 2007; Mayack & Naug 2009; Naug & Gibbs 2009). At the colony level, *Nosema* infection can negatively impact on colony strength and productivity (*e.g.* colony size, brood rearing and honey production; Farrar 1947; Anderson & Giacon 1992; Botías *et al.* 2013). While *N. apis* is a long-known parasite of *A. mellifera*, it was recently demonstrated that *N. ceranae* switched host from the Asian honeybee *A. cerana* to the western honeybee *A. mellifera* (Fries 2010) with an apparent higher virulence than the natal fungal pathogen *N. apis* (Paxton *et al.* 2007). As an emergent pathogen of *A. mellifera*, *N. ceranae* has been suggested to lead to colony collapse in Mediterranean regions (Higes *et al.* 2008, 2009), whereas it has been dismissed as a cause of colony losses under temperate climates (*e.g.* Genersch *et al.* 2010; Paxton 2010; Dainat *et al.* 2012). Since climate may influence *N. ceranae* virulence, its impact at the colony level varies between geographical locations.

Honeybees are particularly sensitive to a large range of chemical insecticides (Stefanidou *et al.* 2003; Thompson 2003; Barnett *et al.* 2007) due to a relative deficit of detoxification enzymes (Yu *et al.* 1984; Claudianos *et al.* 2006) and are exposed to a cocktail of pesticides used in

agricultural (*e.g.* neonicotinoids) and hive pest control (Rosenkranz *et al.* 2010). Recent analyses of pollen from managed bees located near agricultural crops have reported that many agrochemicals such as insecticides, miticides, fungicides and herbicides, can accumulate in comb wax and pollen samples to very high levels (Mullin *et al.* 2010; Wu *et al.* 2011). Among the many compounds detected, neonicotinoids have received the most attention.

Neonicotinoids were developed in the 1980s, and the first commercially available compound, imidacloprid, has been in use since the early 1990s (Kollmeyer *et al.* 1999). Despite it is not clearly established whether neonicotinoids have contributed to yield increases in farming or wether neonicotinoids offer economic benefit compared to alternatives, their use has grown considerably (Blaquière *et al.* 2012; Goulson 2013). Their advantages of low toxicity to vertebrates, high toxicity to insects, flexible use and systemic activity led to neonicotinoids swiftly becoming the most widely used class of any other insecticides globally (Goulson 2013). Neonicotinoid represent a global market share of 24% (80% of the worldwide insecticide seed treatment market; Jeschke *et al.* 2011). Their widespread use results in residual accumulation of low concentrations in the environment (Mullin *et al.* 2010; Goulson 2013). The systemic properties of such compounds imply many possible exposure pathways to honeybees and pollinators in general (Krupke *et al.* 2012; van der Sluijs *et al.* 2013). Neonicotinoids can be classified into one of three chemical groups, the *N*-nitroguanidines (imidacloprid, clothianidin, thiamethoxam, dinotefuran), nitromethylenes (nitenpyram) and *N*-cyanoamidinest (thiacloprid and acetamiprid; Jeschke *et al.* 2011). Acting as agonists on the nicotinic acethylcholine receptors (nAChRs) of the insect central nervous system (Matsuda *et al.* 2001), their presence leads to hyperactivity of the neuronal system (Tomizawa & Casida 2005; Belzunces *et al.* 2012). In the late 1990s neonicotinoids came under increasing scrutiny over their environmental impact. Thus, numerous studies were performed to assess whether neonicotinoids could be harmful to bees, with particular attention to the most toxic group (*i.e. N*-nitroguanidines). Both lethal and sub-lethal effects have been repeatedly reported, including impaired mobility, memory, communication and flight navigation (*e.g.* Bortolotti *et al.* 2003; Desneux *et al.* 2007; Gross 2008; Decourtye & Devillers 2010; Gill *et al.* 2012; Henry *et al.* 2012; Whitehorn *et al.* 2012). This body of work has galvanized public concern over bee welfare, and led to a ban on the use of the three most common neonicotinoids (*i.e.* imidacloprid, clothianidin, thiamethoxam) on pollinator attractive crops by the European Union (2013). The moratorium has been criticized for being based on weak evidence, particularly because effects have mostly been measured on bees that have been artificially fed with neonicotinoid concentrations that

exceed the levels found in nectar and pollen (Dicks 2013; Carreck & Ratnieks 2014; Godfray *et al.* 2014).

Beekeeping has a crucial role in the conservation of the honeybee population, more particularly in Europe, where local wild communities have vanished (De la Rúa *et al.* 2009; Moritz *et al.* 2010). Any reduction in beekeeping activity will therefore cause a decline of managed honeybee colonies, as already seen in both the USA and Europe (Ellis *et al.* 2010; Neumann & Carreck 2010; Potts *et al.* 2010b). Moritz & Erler (2016) recently attributed honeybee decline in industrialised countries to the decline in beekeeping activity and the increase of honey trade rather than to pathogens, pests or pesticides. Nevertheless, unpredictable colony health due to honeybee diseases can also contribute to the decline of apiculture as hobbyist beekeepers and professionals abandon their beekeeping activities (Moritz *et al.* 2010). In such financial uncertainty, it is also difficult to recruit a new generation of beekeepers.

One crucial factor contributing to the unpredictability of colony health and, by extension, to the decreasing numbers of beekeepers and honeybee colony, is undoubtedly the *Varroa* mite (Boecking & Genersch 2008; Rosenkranz *et al.* 2010). In temperate climates, infested colonies may die within two years without treatment (Rosenkranz *et al.* 2010). Thresholds for economic damage and for irreversible colony damage exist and depend on several factors (*i.e.* mite infestation level, honeybee adult and brood populations, season and viral infection; Delaplane & Hood 1999; Currie & Gatien 2006). Therefore, *Varroa* control strategies have been developed and integrated into beekeeping practices to keep infestation levels below the damage threshold and to prevent colony loss engendered by the mite (Delaplane & Hood 1999). These methods are mainly based on the use of acaricides and may have grave consequences for the colony (reviewed in Rosenkranz *et al.* 2010). Some of them can accumulate within bee products (Bogdanov 2006; Martel *et al.* 2007), select for *Varroa* mites strains resistant to effective acaricides (Sammataro *et al.* 2005), and may cause damages to bees (Johnson *et al.* 2009). Most importantly, by artificially controlling the mite population, the selective pressures that may establish a stable host-parasite relationship are supressed (Fries & Camazine 2001). In response to the many disadvantages accompanying the use of chemical treatment for *Varroa* control and the unavoidable colony losses due to varroosis, breeding programs were forced to adapt by developing research on mite resistance (Büchler *et al.* 2010; Rinderer *et al.* 2010).

Over the last decades, the evolution of beekeeping practices has resulted in the development of techniques that impact and may decrease honeybee colony vitality. In the

majority of breeding programs, traits of apicultural interest (*e.g.* honey yield, swarming and temperament) were selected while traits related to vitality (*e.g.* disease resistance, viability, and adaptation to local conditions) were considered secondary because alternatives were available to compensate deficiencies (*e.g.* acaricide treatment against *Varroa*; Büchler *et al.* 2010; Meixner *et al.* 2010; Costa *et al.* 2012). Non-native commercially more interesting subspecies (*i.e. Apis mellifera carnica*, *Apis mellifera ligustica* and *Apis mellifera caucasica*) have been deliberately introduced and propagated to the detriment of native populations (*i.e. Apis mellifera mellifera*; Maul & Hähnle 1994; Garnery *et al.* 1998a, 1998b; Jensen *et al.* 2005; Strange *et al.* 2008). As a result, managed European honeybee populations have reduced or lost their genetic diversity in comparison to wild African populations (Moritz *et al.* 2007; Jaffé *et al.* 2010). Moreover, large-scale queen breeding, in which most breeders produce and distribute many offspring from a few mother queens, has also exacerbated the reduction in genetic diversity (Büchler *et al.* 2010). Such beekeeping practices lead to a large-scale genetic homogenisation and subsequently to the loss of traits, long-shaped by natural selection, involved in local adaptations (Strange *et al.* 2007; Costa *et al.* 2012; De la Rúa *et al.* 2013). Considering the importance of genetic variability to honeybee vitality (Tarpy 2003; Jones *et al.* 2004; Mattila & Seeley 2007) breeding practices, as described, are detrimental to the conservation of the genetic diversity of the endemic honeybee subspecies.

Some European populations surviving mite infestation for long periods without treatment have been reported in different parts of the world (Kurze *et al.* 2016). Those populations offer a good opportunity for breeders to identify and select for resistant-related traits to produce mite-tolerant strains of European honeybees (Büchler *et al.* 2010). They have had some success, most notably with bees expressing the *Varroa*-sensitive hygiene trait (Harbo & Harris 2005; Ibrahim & Spivak 2006). However, the resistance mechanisms are complex and are still only partially understood. Furthermore, resistance does not occur as an isolated interaction between a host colony and its parasite, but depends on hive management and environmental conditions including other pathogens, factors which must be considered for successful resistance selection.

Beekeepers tend to keep colonies locally at an extremely high colony density. This is convenient and practical from a management perspective, but it may also have detrimental consequences at both the apiary and population levels. Aggregation of colonies facilitates the spread of bee diseases by increasing both drifting and robbing behaviours (Free 1958; Fries & Camazine 2001). In large-scale commercial migratory beekeeping, considerable numbers of hives are transported from different regions to areas corresponding to commercial crops. The

greatest agricultural pollination event in the world occurs in the Central Valley of California during the blooming season, where more than 60% of the commercially managed honeybee colonies of the United States (~1.6 million) are required (Brutscher *et al.* 2016). By maintaining numerous hives close to each other and translocating them over a long distance, migratory beekeeping puts bees at risk to encounter and disseminate pathogens (Welch *et al.* 2009). Welch *et al.* (2009) reported higher pathogen prevalence and greater rates of multiple infections in migratory bees than local bees. An exceptionally high colony abundance inevitably boosts inter-colony pathogen transmission through drifting, but also inter-individual transmission through more frequent interactions at resources (flowers, water) or, for drones, at DCAs (Fries & Camazine 2001).

Considering the crucial role of beekeeping activity in maintaining honeybee populations and all the biodiversity that relies on it, precautious in honeybee management need to be rigorously taken to reduce the impact of the current threats to honeybees' vitality (*i.e. Varroa* mite, viruses, *Nosema* spp., neonicotinoids). Understanding the resultant interactions among pathogens, pesticides and management is essential to the comprehension of colony losses and the development of sustainable strategies for promoting colony vitality (Moritz *et al.* 2010).

Study questions

As the current decline of wild and managed honeybees has been linked to beekeeping, pathogens and the use of neonicotinoids, the aim of this thesis is to investigate their impacts on honeybee colony vitality. More specifically, the first part of the thesis will explore the influence of high colony density generated by honeybee management on virus prevalence, and the influence of pathogens on drifting, the major intracolonial transmission path in managed colonies, in an apiary base setting. Given the many detrimental effects of neonicotinoids on honeybees that have been recently reported, the second part of this thesis considers their impacts on both queen mating and social coherence. Since queen mating is paramount within-colony genetic diversity and social coherence is crucial for colony functioning and maintenance, they both have major implications for colony vitality. The following major questions are addressed in each of the next four chapters:

- I. Does high colony abundance induce a higher prevalence of honeybee pathogens?
- II. Do honeybee pathogens facilitate their transmission by enhanced drifting of workers from one colony to another?
- III. Do neonicotinoids impact on honeybee queen mating?
- IV. Do neonicotinoids disrupt the social coherence in groups of honeybee workers?

Chapter I . Viral prevalence increases with regional colony abundance in honey bee drones (*Apis mellifera* L.)

N. Forfert, M.E. Natsopoulou, R.J. Paxton and R.F.A. Moritz

Transmission among colonies is a central feature for the epidemiology of honey bee pathogens. High colony abundance may promote transmission among colonies independently of apiary layout, making colony abundance a potentially important parameter determining pathogen prevalence in populations of honey bees. To test this idea, we sampled male honey bees (drones) from seven distinct drone congregation areas (DCA), and used their genotypes to estimate colony abundance at each site. A multiplex ligation dependent probe amplification assay (MLPA) was used to assess the prevalence of ten viruses, using five common viral targets, in individual drones. There was a significant positive association between colony abundance and number of viral infections. This result highlights the potential importance of high colony abundance for pathogen prevalence, possibly because high population density facilitates pathogen transmission. Pathogen prevalence in drones collected from DCAs may be a useful means of estimating the disease status of a population of honey bees during the mating season, especially for localities with a large number of wild or feral colonies.

Chapter II . Parasites and pathogens of the honeybee *(Apis mellifera)* and their influence on inter-colonial transmission

N. Forfert, M.E. Natsopoulou, E. Frey, P. Rosenkranz, R.J. Paxton and R.F.A. Moritz

Pathogens and parasites may facilitate their transmission by manipulating host behavior. Honeybee pathogens and pests need to be transferred from one colony to another if they are to maintain themselves in a host population. Inter-colony transmission occurs typically through honeybee workers not returning to their home colony but entering a foreign colony ("drifting"). Pathogens might enhance drifting to enhance transmission to new colonies. We here report on the effects infection by ten honeybee viruses and Nosema spp., and Varroa mite infestation on honeybee drifting. Genotyping of workers collected from colonies allowed us to identify genuine drifted workers as well as source colonies sending out drifters in addition to sink colonies accepting them. We then used network analysis to determine patterns of drifting. Distance between colonies in the apiary was the major factor explaining 79% of drifting. None of the tested viruses or Nosema spp. were associated with the frequency of drifting. Only colony infestation with Varroa was associated with significantly enhanced drifting. More specifically, colonies with high Varroa infestation had a significantly enhanced acceptance of drifters, although they did not send out more drifting workers. Since Varroa-infested colonies show an enhanced attraction of drifting workers, and not only those infected with Varroa and its associated pathogens, infestation by Varroa may also facilitate the uptake of other pests and parasites.

Chapter III . Neonicotinoid pesticides can reduce honey bee colony genetic diversity

N. Forfert, A. Troxler, G. Retschnig, L. Gauthier, L. Straub, R.F.A. Moritz, P. Neumann and G.R. Williams

Neonicotinoid insecticides can cause a variety of adverse sub-lethal effects in bees. In social species such as the honeybee, *Apis mellifera*, queens are essential for reproduction and colony functioning. Therefore, any negative effect of these agricultural chemicals on the mating success of queens may have serious consequences for the fitness of the entire colony. Queens were exposed to the common neonicotinoid pesticides thiamethoxam and clothianidin during their developmental stage. After mating, their spermathecae were dissected to count the number of stored spermatozoa. Furthermore, their worker offspring were genotyped with DNA microsatellites to determine the number of matings and the genotypic composition of the colony. Colonies providing the male mating partners were also inferred. Both neonicotinoid and control queens mated with drones originating from the same drone source colonies, and stored similar number of spermatozoa. However, queens reared in colonies exposed to both neonicotinoids experienced fewer matings. This resulted in a reduction of the genetic diversity in their colonies (i.e. higher intracolonial relatedness). As decreased genetic diversity among worker bees is known to negatively affect colony vitality, neonicotinoids may have a cryptic effect on colony health by reducing the mating frequency of queens.

Chapter IV. Thiacloprid alters social interactions among honeybee workers (*Apis mellifera*)

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Experiments have shown that sublethal doses of neonicotinoids can interfere with honey bee (*Apis mellifera*) performance, yet sublethal effects on an individual level may be either enhanced or buffered against at the colony level, and this response to pesticide exposure depends on how it affects worker-worker interactions. We quantified worker interactions in experimental groups to assess the effects of thiacloprid on social network structure established by a group of worker individuals. We also quantified the amount of food exchanged via trophallaxis among worker individuals. Bees were force-fed a "low" dose of $0.17\,\mu g$ or a "high" dose of $0.80\,\mu g$ thiacloprid in $20\,\mu l$ 2.7 M sucrose solution. Bees fed with thiacloprid significantly reduced their network centrality, but they nevertheless exchanged more food to other group members, which resulted in a dilution of the contaminated food. Hence, although thiacloprid may act as a general perturbator of social network structure, it still may play a role in the dynamics of disease transmission in the colony if pathogens are transmitted via food exchange.

General discussion

The Western honeybee *A. mellifera* is arguably one of the most important beneficial insects to humankind and nature through the honey they produce, the crops they pollinate and the employment they provide (Southwick & Southwick 1992; Williams 1994; Roubik 1995; Klein *et al*. 2007; De la Rúa *et al*. 2009). Their pollination service makes them essential for the maintenance of wild flora biodiversity and global food production (Williams 1994; Roubik 1995; Klein *et al.* 2007). The recent decrease in the number of managed honeybee colonies in both Europe and the United States (Ellis *et al.* 2010; Moritz *et al.* 2010), in addition to the high overwinter loss of colonies (vanEngelsdorp & Meixner 2010), have raised concerns about the fate of honeybees and all the species which rely on them. Even though a decreasing number of managed colonies seems to result from socio-economic factors (Neumann & Carreck 2010; Moritz & Erler 2016), sudden losses of honeybee colonies are multifactorial, including pests, pathogens, neonicotinoids, beekeeping practices, and their interactions (*e.g.* Higes *et al.* 2006; Desneux *et al.* 2007; Oldroyd 2007; Neumann & Carreck 2010; Potts *et al.* 2010b; vanEngelsdorp & Meixner 2010; Williams *et al.* 2010; Le Conte *et al.* 2012; Martin *et al.* 2012; González-Varo *et al.* 2013; Goulson *et al.* 2015). Thus, efforts to gain insight into the understanding of factors driven colony vitality, *i.e.* the capacity of a colony to adapt to a local environment at a given time, are paramount for the comprehension of colony losses. The aim of this thesis is to investigate the influence of beekeeping practices, pathogens and neonicotinoid insecticides on honeybee vitality.

One major consequence of honeybee management is the clustering of colonies which increases colony density higher than in natural settings. Honeybees living in a high colony density experience greater competition for resources than honeybees living in a lower colony density. In such conditions, forarger-forarger interactions at food resources are more frequent and food shortage is more likely to happen, increasing the risk for a colony to be robbed (Free 1955). Also, the proximity of resembling hives within the apiary is a factor known to enhance drifting behaviour, as demonstrated in **Chapter II**.

Drifting is the main inter-colonial transmission path within managed colonies (Fries & Camazine 2001), so beekeepers have developed strategies to lower honeybees' orientation mistakes to compensate for the extreme colony density. The idea is to provide visual cues to help honeybees recognise their colonies, by using different hive colours, differently orientating hive entrances or by adopting a specific apiary layout (*e.g.* Free & Spencer-Booth 1961; Cooke

1962). Despite the efforts of beekeepers, they are often insufficient to overcome the effect of the artificially high colony density in such a context (Jay 1968; Pfeiffer & Crailsheim 1998).

In regions with high colony density, the *Varroa* population dynamic is influenced by the continuous exchange of mites through drifter or robber bees (Goodwin *et al.* 2006; Frey & Rosenkranz 2014). Consequently, *Varroa* and associated pathogens may benefit from a high colony density which promotes their transmission. Also, in **Chapter II** it was demonstrated that the mite is not only a passive actor in its inter-colonial transmission but it is also able to influence the drift of worker at individual and colony levels. Workers from highly *Varroa*-infested colonies were more likely to drift than workers from lower infested colonies. Thus, *Varroa* contributes significantly to drifting either by impairing workers' orientation returning from flights, or by inducing this behaviour to enhance its inter-colony transmission (Schmid-Hempel 1998). These results are in concordance with Kralj & Fuchs (2006) who reported that the *Varroa* mite impairs the homing efficiency of foragers. Furthermore, colonies with a high *Varroa* infestation rate showed a higher acceptance of drifters, probably due to an impaired ability to scrutinise incoming foreign workers by guard bees (Annoscia *et al.* 2015).

Varroa also acts as vector for several honeybee pathogens, especially for the most prevalent DWV-family (Kevan *et al.* 2006), so one may expect a higher pathogen prevalence at high rather than at low colony densities. This hypothesis is validated in **Chapter I**. Indeed, there was a positive association between colony density and virus prevalence in drones, both at the individual and the colony level. More specifically, drones from high density sampling sites show a higher probability of DWV-family infections and of multiple viral infections than drones from low density, most likely due to increased pathogen transmission through higher rates of drifting, robbing and worker-worker interactions (Betts 1932; Bailey 1958; Free & Spencer-Booth 1961; Jay & War 1984; Fries & Camazine 2001). These results are in concordance with the previous findings of Welch *et al.* (2009) who reported higher pathogen prevalence and greater rates of multiple infections in migratory bees than in local bees. Despite no direct effect of *Varroa*-associated viruses on drifting behaviour being found in **Chapter II**, the prevalence of *Varroa*-transmitted viruses was positively associated with a high colony density in **Chapter I**. This suggests that increased *Varroa*-associated virus prevalence at the population level in high colony density settings may be a consequence of enhanced drifting caused by their *Varroa* vector at individual and apiary levels. Hence, any increased transmission through drifting workers induced by *Varroa* would not only be beneficial to the mite itself, but also to the pathogens it carries. Nevertheless, *Varroa* infested colonies are likely to acquire more pathogens since their probability of receiving drifters and hence, also pathogens, is greater

than in colonies with low *Varroa* infestation. These colonies will therefore eventually serve not only as sources of pathogens for healthy honeybee colonies, but also for other wild pollinators (McMahon *et al.* 2015). With high honeybee colony densities, honeybees and other pollinators are more likely to interact with each other due to increased competition for food resources. Since honeybee-infecting viruses have also be found to infect other pollinator species (*e.g.* bumble bees), a high colony density may facilitate the spread of those viruses to non-*Apis* species via shared food sources (Singh *et al.* 2010; Fürst *et al.* 2014; McMahon *et al.* 2015). Moreover, diseases transmission is not the only threat to other pollinators generated by honeybee management, Elbgami *et al.* (2014) demonstrated that bumblebee colonies located close to a large honeybee apiary exhibited lower fitness than more distant colonies, most likely due to greater resource competition among honeybees and bumblebees. Since wild pollinators such as bumblebees are in global decline (Williams & Osborne 2009), an extremely high density of managed honeybees may be detrimental to their conservation. Therefore, all efforts to maintain managed honeybees at a low density is recommended as it is not only of benefit to the honeybees but also to pollinators in general.

Taking together the findings of **Chapters I** and **II**, and since *Varroa* infestation can be controlled by adapted treatments, it appears that the main factors affecting the dynamic of the inter-colonial transmission of pests and pathogens (*i.e.* colony density, inter-hive distance and *Varroa* infestation) depend greatly on beekeepers. The apiary layout and high colony density facilitates inter-colonial transmission, and uncouples the trade-off between virulence and transmission typically seen for pathogens and parasites (Schmid-Hempel 2011). This is true for both the mite and the pathogens it transmits. Efficient treatment against *Varroa* is crucial, not only to help beekeepers to maintain a *Varroa* infestation ratewell below the *Varroa* damage threshold (Currie & Gatien 2006) causing colony loss during winter (Genersch *et al.* 2010), but also to reduce horizontal transmission of pests and associated pathogens among colonies. Nevertheless, because of the various problems posed by acaricide-based *Varroa* treatments (reviewed in Rosenkranz *et al.* 2010), particularly those regarding the suppression of the selective pressures necessary for the establishment of a stable host-parasite relationship (Fries & Camazine 2001; Schmid-Hempel 2011), the only sustainable strategy to control the mite is breeding *Varroa*-resistant bees. For this reason, efforts to produce mite resistant lines of European honeybees for commercial use is of major concern for many bee researchers and bee breeders (see "SmartBees" project: http://www.smartbees-fp7.eu/).

Even if bee breeding seems promising in term of *Varroa* control, resistance mechanisms are complex and are still only partially understood. *Varroa* resistance, and pathogens resistance

in general, do not simply occur as an isolated interaction between the honeybee host colony and the mite. Rather, it depends on a broad range of additional factors that may interact synergistically such as local management and environment conditions, and other pathogens (*e.g.* viruses) which must be considered while designing selection resistance programs. Additionally, it is worth mentioning that all the reported *A. mellifera* populations which have developed adaptations to *Varroa* infestation are unmanaged (Locke 2015). Those *Varroa* mite tolerant honeybee populations provide excellent opportunities to study genetic and ecological factors that enable mite resistance including important mite-resistant traits that could be adopted in breeding programs. As an example, Behrens *et al.* (2011) screened the genome of drones from a mite-surviving population to identify quantitative trait loci (QTLs) possibly involved in the inhibition of mite reproduction. Their analysis found target regions on three chromosomes with QTL that seemed to interfere with mite reproduction (Behrens *et al.* 2011). In a follow-up study, Lattorff *et al.* (2015) scanned these QTL regions in samples of bees from the same population before and after natural selection had occurred. They found a strong overall loss of heterozygosity in these regions, suggesting that genetic drift, selection, or both had occurred in the population.

Colony vitality does not only relate to diseases prevailing in an environment, but also to its capacity to adapt in general to regional environmental factors such as pesticides (Meixner *et al.* 2010). Although there is little convincing evidence for direct mortality in honeybees, there is strong evidence for important sublethal effects. Exposure to sublethal doses of neonicotinoids is known to affect cognition (*e.g.* learning, memory, sense perception), behaviour (*e.g.* mobility, homing) and physiology in bees (*e.g.* muscle activity, immunity, thermoregulation) (*e.g.* Bortolotti *et al.* 2003; Medrzycki *et al.* 2003; Desneux *et al.* 2007; Gross 2008; Aliouane *et al.* 2009; Decourtye & Devillers 2010; Belzunces *et al.* 2012; Blacquière *et al.* 2012; Gill *et al.* 2012; Henry *et al.* 2012; van der Sluijs *et al.* 2013). The impact of neonicotinoid insecticides on two crucial aspects of colony vitality, *i.e.* queen mating success (**Chapter III**) and within-group interaction (**Chapter IV**), is the focus of the second part of this thesis.

At the colony level, genetic variability generated by multiple mating has been shown to be important for disease resistance, homeostasis, thermoregulation and overall colony fitness (Tarpy 2003, Jones *et al.* 2004; Seeley & Tarpy 2007; Mattila & Seeley 2007). Thus, genetically diverse colonies are more likely to adapt and respond to environmental constraints than genetically homogenous colonies.

Within the colony, thousands of individuals form a complex network resulting from communication and self-organisation to eventually produce a group-level response (Fewell

2003). Within dense networks of interacting individuals, information is distributed rapidly, allowing them to elaborate a flexible and efficient response to the dynamic of the environment in which they live (Fewell 2003). Thus, fully functional communication is fundamental for colony vitality since it interferes in every aspect of colony functioning (Bortolotti & Costa 2014).

The results found in **Chapters III** and **IV** suggested that neonicotinoids may have cryptic but potentially grave consequences on colony vitality by lowering the frequency of queen mating, and by disrupting the social network of interactions. Since genetic variability may facilitate worker complementation and hence colony efficiency, the impact of neonicotinoids on both the queen mating frequency and social network of communication may act synergistically to the detriment of colony functioning and resilience. The synergic effect of reduced genetic diversity and communication may represent an important threat and could be aggravated in colonies already suffering from other stressors. For example, using an observation hive, Annoscia *et al.* (2015) showed that *Varroa* infested bees exhibited reduced activity and participation to hive duties (*e.g.* trophallactic interactions). The authors (Annoscia *et al.* 2015) assumed that the mite reduces the activity rate of infested individuals. Therefore, the lack of bees involved in colony functions added to the disruption in social communication, quickly leading to the dysfunction of the entire colony with adverse impacts on colony vitality. The loss of genetic diversity caused by neonicotinoids may be of additional concern for intensively managed breeding populations in Europe which may have already suffered severe loss of genetic diversity due to rigorous selection in breeding programs (Moritz *et al.* 2007). Thus, the combined effects of both neonicotinoids and overly rigid breeding programs may lead to irreversible genetic homogenisation and the subsequent loss of traits which are essential for local honeybee colony vitality.

Chapter III and **IV** focused on different groups of neonicotinoid compounds: the *N*-nitroguanidines (*i.e.* clothianidin, thiamethoxam) and the *N*-cyanoamidinest (*i.e.* thiacloprid), which differ greatly on many aspects. Firstly, the nitro-substituted neonicotinoids appear the most toxic to bees with low oral LD_{50} (i.e. the dose that kills 50% of individuals within a certain time; LD_{50} = 0.005 and 0.003 µg/bee for thiamethoxam and clothianidin, respectively; Decourtye & Devillers 2010). In contrast, thiacloprid, a cyano-substituted neonicotinoid, is considered one of the less toxic neonicotinoid compound (oral LD_{50} = 17.32 µg/bee; Decourtye & Devillers 2010; Laurino *et al.* 2011). Therefore, a broad range of studies investigating the impact of the nitro-substituted neonicotinoids group are available (*e.g.* Aliouane *et al.* 2009; Decourtye & Devillers 2010; Retschnig *et al.* 2015; Williams *et al.* 2015) with less attention to

the impact of thiacloprid on honeybees. Secondly, the mode of application differs from both groups. The nitro-substituted group is routinely used as seed dressing of major forage sources (*e.g.* oilseed rape, sunflower and maize) for both managed honeybees and wild pollinators (Goulson 2013). Because of their systemic properties, neonicotinoids are found in both pollen and nectar of seed-treated crops. In contrast, thiacloprid is rather routinely applied as foliar sprays to fruit crops (*e.g.* raspberries), which are visited by both managed and wild pollinators. Thus, widespread but unquantified use of thiacloprid as foliar spray provides a direct route of exposure for pollinators. Those differences in use and impacts highlitght the complexicity poses by the study of neonicotinoid effect in general.

From an epidemiological perspective, conclusions on the combined effects of neonicotinoids on queen mating and social interactions on the dynamic of disease transmission are arduous to draw since neonicotinoids have opposite effects on those colony functions. Indeed, on the one hand, extreme polyandry contributes to increased resistance towards pests and pathogens of the colony (Tarpy 2003; Tarpy & Seeley 2006; Seeley & Tarpy 2007; Delaplane 2015), probably due to more efficient hygienic and grooming behaviour. Thus, lower genetic diversity induced by thiamethoxam and clothianidin (**Chapter III**) may lead to higher sensitivity towards pests and pathogens. On the other hand, results from **Chapter IV** suggest that workers exposed to thiacloprid tend to interact less with other nestmates through antennation, while they shared more food via trophallaxis. Since social interactions shape the dynamic of pathogen transmission between individuals, neonicotinoids may potentially impair this dynamic, except in the case of food-borne pathogens (*e.g. Nosema*; Higes *et al.* 2010). Also, parasites can alter host behaviour to enhance their own transmission to new susceptible hosts, as seen with *Varroa* in **Chapter II**. In addition, the microsporidian gut parasite *Nosema* has been shown to reduce interactions among workers (*i.e.* trophallaxis; Naug & Gibbs 2009). *Nosema* infections can turn workers into trophallactic sinks and decrease the connectivity of social networks within the colony. This potential strategy to reduce the transmission of the parasite may be compromised if honeybees become momentarily poisoned by pesticides. Thus, the role of neonicotinoids on the dynamic of pest and pathogen transmission can be expected to be highly complex and most likely, pathogen specific. Further studies may be rewarding to investigate the interactions of neonicotinoids on genetic diversity and parasite transmission.

In a more general aspect, neonicotinoids do not only represent a potential threat to honeybees but may impact on a broad range of non-target taxa including wild pollinators, soil and aquatic invertebrates and hence threatens a range of ecosystem services (Desneux *et al.* 2007; Goulson 2013; Rundlöf *et al.* 2015). The prophylactic use of neonicotinoids as seed

dressings goes against the principles of integrated pest management (IPM). IPM is a broad-based approach that predicates on minimizing use of chemical pesticides via monitoring of pest populations, making maximum use of biological and cultural controls, applying chemical pesticides only when needed and avoiding broad-spectrum, persistent compounds (Metcalf & Luckmann 1994). IPM minizes pesticide use, reduces the likelihood of the development of resistance in pests and minimizes impacts on non-target organisms. The prophylactic application of neonicotinoid, their persistence in soil and water make them bioavailable to bees and other pollinators at sublethal concentrations for most of the year. Therefore, it is not surprising that the prophylactic use of such chemicals has led to some insect pests developing resistance (e.g. Horowitz *et al.* 2004; Szendrei *et al.* 2012). Despite a broad range of studies have investigated the impact of neonicotinoid on honeybees and bumblebees (*e.g.* Gill *et al.* 2012; Whitehorn *et al.* 2012), there are still knowledge gaps concerning possible impacts on pollinators other than bees (Rundlöf *et al.* 2015). There is clear evidence of recent declines in both wild and domesticated pollinators in some industrialized countries and parallel declines in the plants that rely upon them (Potts *et al.* 2010a; van der Sluijs *et al.* 2013). Thus, there is an urge to understand to which extend neonicotinoids contribute to pollinator declines that could significantly affect the maintenance of wild plant diversity, wider ecosystem stability, crop production, food security and human welfare.

All factors affecting colony vitality which were investigated in this thesis (*i.e.* beekeeping practices, pests and pathogens, and neonicotinoids) seem to be directly or indirectly connected to each other. The additive effects of those stressors affecting different vitality-related traits can inevitably lead to grave consequences for the colony, and more generally, to the conservation of honeybees and all the biodiversity which depends on their pollination service. Thus, it is not surprising that no major factor for honeybee colony losses has been identified, rather, this phenomenon depends on a multitude of interconnected factors.

Conclusion and future challenges

The fate of honeybees and their reliant biodiversity is of major concern to all. Even though sudden losses of honeybee colonies directly compromise beekeeping activity, clearly honeybee management itself has played a central role in this phenomenon, especially by interfering in the coevolution process between honeybee host colony and parasite (Fries & Camazine 2001). This places beekeepers at a pivotal position for the conservation of honeybee, particularly in countries where wild and feral populations have completely vanished (Moritz *et al.* 2007; De

la Rúa *et al.* 2009). With regard to the crucial role of beekeeping activity in maintaining honeybee populations and all the biodiversity that relies on honeybee pollination, precautions in their management need to be rigorously taken to reduce the impact of the current threats to honeybee colony vitality. Deeper knowledge of colony vitality and how current beekeeping practices impact on it is necessary to establish long-term sustainable honeybee management strategies in apiculture. Understanding the interactions between pathogens, pesticides and management is essential for the comprehension of colony losses and the development of sustainable strategies for promoting colony vitality (Moritz *et al.* 2010). Also, *Varroa* resistance mechanisms need to be further investigated to develop and improve sustainable control strategies rather than depending on the heavy use of inefficient acaricide treatment.

More than ever, beekeepers and bee researchers need to cooperate to understand the highly complex network of colony vitality stressors. The actual challenge that modern bee breeders face is to maintain the traditional traits related to colony performance described by Ruttner (1972) which are considered of major apicultural interest, while incorporate traits related to colony vitality. Strategies for better beekeeping practice that can reduce pathogen virulence, by inhibiting the critical infection pathways that management otherwise induces, are urgently needed. A profound reorganisation of beekeeping activity is necessary with disease awareness education to reduce the impact on honeybee colony vitality, but also on other threatened pollinators. Furthermore, since the loss of managed colonies is tightly linked to the reduction in the number of beekeepers, policies should be reconsidered to encourage professional and hobbyist beekeepers to maintain and develop their activities (Neumann & Carreck 2010; Moritz & Erler 2016), supporting them to overcome the transition to more sustainable practice.

With regards to the use of neonicotinoids, studies have suggested that their widespread and increasing use poses a particular threat to honeybee colony vitality and other pollinators which impacts pollination services and may be playing a role in driving biodiversity loss (*e.g.* Goulson *et al.* 2013). The recent partial restriction on the use of neonicotinoids by the European Commision (2013) and the tension between the agricultural and environmental consequences, has made this topic one of the most controversial involving science and policy. The key questions are how field-realistic doses received by pollinators influence individual performance, and whether the cumulative effect on colonies and populations affects pollination and the conservation of pollinator populations (Vanbergen 2013). Thus, there is an urge to find a trade-off between agricultural and biodiversity outcomes. The current use of neonicotinoids needs to be re-evaluated to assess whether it provides the optimum balance between meeting

the demands of food production and farming profitability, vs. the need to sustainably manage global biodiversity to ensure vital ecosystem services upon which all life depends (Goulson 2013). Agricultural landscapes show a wide range of ecological conditions and biodiversity which depend on a combination of factors (*e.g.* climate, soil condition, water availability, intensity and scale of management). Therefore, reconciliation between agriculture and biodiversity conservation has to be adapted to the local region involved, according to the species and habitats as well as the particular socio-economic characteristics (Henel *et al.* 2008). Such reconciliation requires the participation of local stakeholders to guarantee its success and sustainability (Henel *et al.* 2008).

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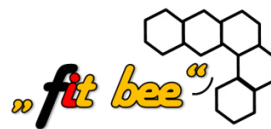
I would like to thank all the people and good friends I met in Halle who contributed to make my stay unforgettable, the French connection: Alexis and Bertrand, Maria, Sandrine, Rawy, John, Christoph, Myrsini, Panas, Sajjad and all my friends from Papa Dula. I thank also my friends from all around the world who always support me, Panda, Toto, Ryad, Olivier, and all the members of Bonheur d'Enfants d'Afrique, an African association in which I am involved, because they dedicate their lives to help the children in need.

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Appendix A. Supplementary material and data – Chapter II.

Table S.II.1. Viruses and *Nosema* infection, *Varroa* infestation and proportion of honeybee drifters (*Apis mellifera*) detected in acaricides treated or untreated colonies in two different apiaries sites, Kenzingen (K) and Simonswald (S), Germany. At each apiary, seven colonies were treated against *Varroa* and seven were untreated. Honeybee foragers were sampled flying back to the hive after passing the guarding bees. Among them, individuals were identified as drifters. For some of them, their source colony could not be identified. The level of viruses and *Nosema* infections is based on the infection of the native foraging bees. *Varroa* infestation was determined from an independent sample of 150 in-hive bees.

Site	Colony	Acaricide treatment	Total number of individuals	Number of drifters	Number of drifters from unknown colony source	DWV-family prevalence (%)	BQCV prevalence (%)	CBPV prevalence (%)	Rate of *Varroa* infestation
K	1	No	10	0	-	80	40	0	14.40
K	2	No	10	0	-	30	10	0	10.20
K	3	No	10	0	-	10	10	0	2.77
K	4	No	18	8	1	20	20	0	9.23
K	5	No	17	4	2	38.46	23.08	0	7.03
K	6	No	10	0	-	30	50	0	11.70
K	7	No	11	0	-	0	36.36	9.09	4.77
K	8	Yes	16	6	0	50	50	0	0.27
K	9	Yes	11	2	2	0	22.22	0	0.40
K	10	Yes	9	0	-	11.11	55.55	0	0
K	11	Yes	9	0	-	0	33.33	0	0.60
K	12	Yes	10	0	-	10	20	0	0
K	13	Yes	10	0	-	0	30	0	0
K	14	Yes	10	0	-	0	20	0	0.40
S	15	No	17	14	9	0	0	0	0.60
S	16	No	11	2	1	0	33.33	0	2.37
S	17	No	15	8	2	42.86	28.57	0	1.033
S	18	No	14	5	3	44.44	22.22	0	2.67
S	19	No	8	3	0	20	0	0	1.87
S	20	No	17	9	3	0	0	12.50	3.10
S	21	No	10	4	2	0	0	16.67	0.63
S	22	Yes	10	0	-	0	0	0	0.13
S	23	Yes	15	5	0	20	10	10	0.20
S	24	Yes	15	2	2	15.38	0	7.69	0
S	25	Yes	12	1	0	9.09	0	0	0.43
S	26	Yes	7	2	1	60	20	0	0
S	27	Yes	6	0	-	16.67	16.67	0	0
S	28	Yes	10	0	-	10	0	0	0

Appendix B. Declaration on the author contribution

Chapter I. Forfert N, Natsopoulou ME, Paxton RJ, Moritz RFA. Viral prevalence increases with regional colony abundance in honey bee drones (*Apis mellifera* L.). **Infect. Genet. Evol.** 2016, 44, 549–554.

Sampling: 50%

Experimental work: 50%

Data analysis: 70%

Writing: 80%

Chapter II. Forfert N, Natsopoulou ME, Frey E, Rosenkranz P, Paxton RJ, Moritz RFA. Parasites and pathogens of the honeybee (*Apis mellifera*) and their influence on inter-colonial transmission. **PLoS ONE** 2015, 10(10), e0140337

Experimental work: 50%

Data analysis: 70%

Writing: 80%

Chapter III. Forfert N, Troxler A, Retschnig G, Gauthier L, Straub L, Moritz RFA, Neumann P, Williams GR. Neonicotinoid pesticides can reduce honey bee colony genetic diversity. **PLoS ONE** 2017, https://doi.org/10.1371/journal.pone.0186109

Experimental work: 30%

Data analysis: 70%

Writing: 80%

Chapter IV. Forfert N, Moritz RFA. Thiacloprid alters social interactions among honeybee workers (*Apis mellifera* L.). **J. Apicult. Res.** 2017, http://dx.doi.org/10.1080/00218839.2017.1332542

Project design: 80%

Experimental work: 70%

Data analysis 100%

Writing: 80%

Appendix C. Curriculum vitae

Nadège Forfert

Home address:	3 Bis rue Saint-Gengoulf 57070 Metz (France)
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EDUCATION

2012-2018   Ph.D., Molecular Ecology
Martin-Luther Universität Halle-Wittenberg, Germany

2011   M.Sc., Ecology and Evolution of Parasites
Université de Montpellier 2, France

2009   B.Sc., Biology
Université Libre de Bruxelles, Belgium

2005   Intensive English courses
EF school Miami, USA

PROFESSIONAL EXPERIENCES AND INTERNSHIPS

Ph.D. candidate – *March 2012-2018*
> Department of Molecular Ecology, MLU Halle-Wittenberg, Germany
> Research interest: "*Impact of beekeeping practices and neonicotinoids on honeybee colony vitality*"
> Advisor: Prof. Robin FA Moritz

Master Intern – *January 2011-June 2011*
> Research Unit of Malaria, Pasteur Institute of Madagascar, Madagascar
> Research interest: "*Epidemiological school-based study of Plasmodium sp. prevalence in Madagascar and molecular characteristics associated with drug resistance*"
> Advisor: Prof. Milijaona Randrianarivelojosia

Master Intern – *April 2010-July 2010*
> UMR Biology and Genetics of Plant-Pathogen Interaction, CIRAD of Montpellier, France
> Research interest: "*Impact of random homologous recombination in the case of an emerging phytovirus: the Tomato Yellow Leaf Curl Virus*"
> Advisor: Prof. Michel Peterschmitt

ORAL PRESENTATIONS

Forfert N, Moritz RFA. *"Do neonicotinoids affect social network in honeybees?"* 6th European Conference of Apidology (EurBEE), September 8th-11th 2014 in Murcia, Spain (Invited speaker).

Forfert N, Natsopoulou M, Frey E, Rosenkranz P, Paxton RJ, Moritz RFA *"Do pathogens induce honeybee drifting?* Association of the German Bee Research Institutes Conference, March 25th–27th 2014 in Marburg, Germany.

Schluens EA, **Forfert N**, Paniti-Teleky OR, Furdui EM, Dezmirean DS, Moritz RFA *"Using DNA pools for genotyping colonies of the honeybee Apis mellifera with microsatellite DNA."* Association of the German Bee Research Institutes Conference, March 19th –21st 2013 in Würzburg, Germany.

Schluens EA, **Forfert N**, Paniti-Teleky OR, Furdui EM, Dezmirean DS, Moritz RFA *"Using DNA pools for genotyping colonies of the honeybee Apis mellifera with microsatellite DNA."* International Union of the Study of Social Insects Conference, March 14th–18th 2013 in Cluj-Napoca, Romania.

PEER-REVIEWING EXPERIENCE

Referee of papers submitted to the following journals: *Apidologie* and *Archives of Virology.*

Appendix D. Publications

Forfert, N., Troxler, A., Retschnig, G., Gauthier, L., Straub, L., Moritz, R.F.A., Neumann, P., Williams, G.R, 2017. Neonicotinoid pesticides can reduce honey bee colony genetic diversity. https://doi.org/10.1371/journal.pone.0186109

Forfert, N., Moritz, R.F.A., 2017. Thiacloprid alters social interactions among honeybee workers (*Apis mellifera* L.). J. Apic. Res. http://dx.doi.org/10.1080/00218839.2017.1332542.

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Forfert, N., Natsopoulou, M.E., Frey, E., Rosenkranz, P., Paxton, R.J., Moritz, R.F.A., 2015. Parasites and pathogens of the honeybee (*Apis mellifera*) and their influence on inter-colonial transmission. PLoS ONE 10, e0140337. http://dx.doi.org/10.1371/journal.pone.0140337.

Boff, S., **Forfert, N.**, Paxton, R.J., Montejo, E., Quezada-Euan, J.J.G., 2015. A behavioural guard caste in a primitively eusocial orchid bee, *Euglossa viridissima*, helps defend the nest against resin theft by conspecifics. Insect. Soc. 62, 247–249. http://dx.doi.org/10.1007/s00040-015-0397-3.

Vuillaume, F., Thebaud, G., Urbino, C., **Forfert, N.**, Granier, M., Froissart, R., Blanc, S., Peterschmitt, M., 2011. Distribution of the phenotypic effects of random homologous recombination between two virus species. PLoS Patho. 7, e1002028.

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Appendix E. Erklärung

Metz, den 24 Februar 2017

Hiermit erkläre ich an Eides statt, dass diese Arbeit von mir bisher weder an der Naturwissenschaftlichen Fakultät I - Biowissenschaften der Martin-Luther-Universität HalleWittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion eingereicht wurde.

Ich erkläre weiterhin, dass ich mich bisher noch nicht um den Doktorgrad beworben habe.

Ferner erkläre ich, dass ich diese Arbeit selbstständig und nur unter Zuhilfenahme der angegebenen Quellen und Hilfsmittel angefertigt habe. Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen sind als solche kenntlich gemacht worden

Nadège Forfert

Abstract

Colony vitality refers to the adaptability of a colony to a given environment at a given time and comprises both colony health and fitness. As the current decline of wild and managed honeybees has been linked to beekeeping, pathogens and the use of neonicotinoids, the aim of this thesis is to investigate their impacts on honeybee colony vitality. The first part of the thesis explores the influence of high colony density, generated by honeybee management, on virus prevalence, and the influence of pathogens on drifting, the major intracolonial transmission path in managed colonies, in an apiary base setting. Given the many detrimental effects of neonicotinoids on honeybees that have been reported, the second part of this thesis considers their impacts on both queen mating and social coherence. Since queen mating is paramount within-colony genetic diversity, and social coherence is crucial for colony functioning and maintenance, they both have major implications for colony vitality.

Keywords: Drift – Colony density – Beekeeping – Neonicotinoid – Varroa – Nosema – Honeybee viruses – Mating – Social Networks

Abstract

Volksvitalität bezieht sich auf die Anpassungsfähigkeit eines Volkes, an eine gegebene Umgebung zu gegebener Zeit, und umfasst sowohl Volksgesundheit als auch Fitness. Da der aktuelle Rückgang wilder und gemanagter Honigbienen mit Bienenhaltung, Pathogenen und dem Einsatz von Neonicotinoiden in Verbindung gebracht wurde, ist es das Ziel dieser Arbeit, ihre Auswirkungen auf die Vitalität von Bienenvölkern zu ermitteln. Der erste Teil der Arbeit untersucht den Einfluss hoher Völkerdichten (generiert durch Honigbienenmanagement) auf Virenprävalenz, und den Einfluss von Pathogenen auf Verflug (den wichtigsten intrakolonialen Übertragungsweg in bewirtschafteten Völkern), in einer bienenstandsbezogenen Umgebung. In Anbetracht der vielen schädlichen Auswirkungen von Neonicotinoiden auf Honigbienen, werden im zweiten Teil dieser Arbeit die Auswirkungen auf die Paarung und die soziale Kohärenz untersucht. Da die Königinnenpaarung für die genetische Vielfalt innerhalb des Volkes von größter Bedeutung ist und die soziale Kohärenz für das Funktionieren und den Erhalt entscheidend ist, haben beide wichtige Auswirkungen für die Volksvitalität.

Keywords: Verflug – Volksdichte – Bienenhaltung – Neonicotinoid – Varroa – Nosema – Honigbienenviren – Paarung – soziale Netzwerke