Effects of parasites and pathogens on bee cognition

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Abstract. 1. Bees are key pollinators and their widespread decline has raised considerable concerns regarding the sustainability of ecosystems and food production. Many environmental stressors do not directly kill bees, but they alter their physiology and behaviour, ultimately impacting colonies and populations.

2. This review considers the impact of parasites and pathogens on bee cognition.

3. First the main parasites and pathogens of bees are described, as well as how they modify the foraging behaviour, learning and memory of their hosts.

4. Next, the various defence mechanisms developed by bees to mitigate these effects at both the individual and collective levels are examined.

5. Finally, there is a discussion on how integrating research on host parasites, animal behaviour and cognition will provide a more detailed assessment of the contribution of parasites and pathogens to declines in the bee population and help to inform constructive ecological interventions.

Key words. Bumblebees, cognition, *Crithidia*, honey bees, learning and memory, *Nosema*, parasites and pathogens, *Varroa*.

Introduction

Insects face a large diversity of parasites and pathogens, including viruses, bacteria, protozoans, mites, fungi, nematodes and parasitoids (Schmid-Hempel, 1998). These biological antagonists can have a wide diversity of effects on insect physiology, such as pathological lesions, changes in metabolism, homeostasis (e.g. thermoregulation) and physiological functions (e.g. antioxidant activity), as well as challenges to the immune system and alterations of fundamental molecular pathways (e.g. cellular apoptosis) (Beckage *et al.*, 1993; Schmid-Hempel, 2011). In recent years, there has been growing evidence that parasites and pathogens also affect the behaviour of insects, either through a negative impact on their neural system and cognitive abilities (Gegear *et al.*, 2006; Iqbal & Mueller, 2007), or as a response to reduce further risks of contamination (Cremer & Sixt, 2009). In insect species that rely heavily on learning and memory to collect food, any impairment of cognitive functions may have dramatic consequences on individual fitness and ultimately population dynamics.

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Central-place foraging bees, for instance, have evolved remarkable cognitive abilities to efficiently collect nectar and pollen from patchily distributed floral resources in order to provision their brood (von Frisch, 1967; Michener, 2000). Upon leaving their nest, bees must learn to recognise profitable flowers based on visual, olfactory and gustative cues, and to handle them for harvesting food (Menzel, 2012; Giurfa, 2013). Flower discrimination involves various forms of associative learning between a stimulus (or a combination of stimuli) and a reward (nectar, Strang & Sherry, 2014; pollen, Muth *et al.*, 2016). Efficient foraging also includes accurate navigation for relocating familiar food patches (flowers, plants or trees) and developing economical routes between them based on visuo-spatial memories (Collett *et al.*, 2013). A century of research on bee behaviour and cognition, starting with the seminal work on honey bee colour vision by Karl von Frisch (1914), has revealed an unexpectedly rich cognitive repertoire in these animals that sometimes rivals that of larger-brained animals, such as their ability for numerosity (Chittka & Geiger, 1995), mastering concepts (Giurfa *et al.*, 2001), solving combinatorial routing problems (Lihoreau *et al.*, 2012), and acquiring new foraging techniques from observing others (Loukola *et al.*, 2017). In the most social species, such as honey bees, individuals even communicate food locations to their nest mates using a symbolic language (the waggle dance) that encodes the distance and

the direction of discovered resources (von Frisch, 1967; Riley *et al.*, 2005). Using a similar communication system, honey bees make collective decisions by which swarms compare and select the best available nesting sites to establish new colonies (Seeley, 1996; Seeley *et al.*, 2012).

Recently parasites and pathogens have been recognised as major stressors of both wild and managed bees (Goulson *et al.*, 2015), yet their effect on the bee brain and its cognitive abilities are still poorly characterised. In principle, any impairment of cognitive functions may considerably reduce the foraging success of individuals (Klein *et al.*, 2017). Social bees, which live in highly integrated colonies based on division of labour, have evolved cooperative strategies to reduce infection rates and mitigate their effects (Cremer *et al.*, 2007; Cotter & Kilner, 2010). So far, however, defence strategies by solitary bees, which represent the vast majority of bee species (Michener, 2000), are virtually unknown. In these species, a reduced foraging efficiency by females, due to parasites or pathogens, may have direct dramatic consequences for their brood.

In this review, we consider how parasites and pathogens affect the behaviour and cognition of bees. First we describe the main parasites and pathogens known to influence bee physiology and fitness. Next we discuss how many of these biological antagonists impair bee behaviour and cognition, and describe the various behavioural defences bees have evolved to mitigate these effects. We focus on honey bees and bumblebees, two social species that are historical models for experimental research on bees and for which most data are available (Schmid-Hempel, 1998, 2011).

The main parasites and pathogens of bees

Bees are hosts of viruses, protozoans, bacteria, mites, fungi and parasitoid insects that can be transmitted horizontally between individuals of the same generation (e.g. on flowers, mouth-to-mouth food exchanges or during copulation) or vertically into the next generation (e.g. through transovarian transmission, mother to daughter colony; Schmid-Hempel, 1998; Cremer *et al.*, 2007; see details in Table 1).

Microparasites

Honey bees are infected by several viruses, some of which have been linked with population declines. These include the deformed wing virus (DWV; McMahon *et al.*, 2015), the Israeli acute paralysis virus (IAPV) and the acute bee paralysis virus (ABPV; Cox-Foster *et al.*, 2007; Dainat *et al.*, 2012b), which are usually spread by the mite *Varroa destructor* (Tentcheva *et al.*, 2004; Dainat *et al.*, 2012a; Lodesani *et al.*, 2014). Some of them have been found in the honey bee brain tissues, suggesting that bee viruses have an important impact on cognitive functions (Fujiyuki *et al.*, 2009).

Paenibacillus larvae is a spore-forming Gram-positive bacteria responsible for the American Foulbrood disease which can cause winter colony mortality in honey bees (Genersch, 2010). The bacteria colonise the midgut of the larvae where they proliferate, disrupt the epithelium and break down the host into a brown and viscous colloid containing millions of highly resistant spores that contaminate the nest (Genersch, 2010).

Trypanosomatids, such as *Crithidia* spp., have received much attention in bumblebees and, more recently in honey bees. These extracellular parasites attach to the surface of the gut epithelial cells where they reproduce and release new parasitic forms in the faeces (Sadd, 2011; Mcart *et al.*, 2014). *Crithidia bombi* parasitises different bumblebees species and can reduce their reproductive ability and life span (Brown *et al.*, 2003; Otti & Schmid-Hempel, 2008). *Crithidia mellificae* and *Lotmaria passim* are highly prevalent in honey bee colonies (Ravoet *et al.*, 2013; Cepero *et al.*, 2014); however, their pathogenicity is still poorly documented (Schwarz & Evans, 2013; Higes *et al.*, 2016; Stevanovic *et al.*, 2016).

Microsporidia of the genus *Nosema* are obligate parasites of honey bees and bumblebees that invade the ventricular epithelial cells where they reproduce and release spores in the faeces (Fries *et al.*, 1996; Higes *et al.*, 2007; Meeus *et al.*, 2011). *Nosema apis* is the specific microsporidium of *A. mellifera* (Bailey, 1955). *Nosema ceranae* infects *A. mellifera* and other *Apis* (Fries *et al.*, 1996; Chaimanee *et al.,* 2010, 2011) and *Bombus* species (Plischuk *et al.*, 2009; Li *et al.*, 2012). The microsporidia *N. bombi* parasitises bumblebees and reduces the fitness of the reproductive individuals (Otti and Schmid-Hempel 2007).

Macroparasites

The mite *V. destructor* is an obligate ectoparasite of honey bees that infests and weakens both larvae and adults by feeding on their haemolymph. High levels of infestation affect the reproductive capacity of the colony and reduce brood size and the number of adults (Duay *et al.*, 2002; Villa *et al.*, 2008). *Varroa destructor* is also a vector of many honey bee viruses (e.g. DWV) which can further reduce the life span of workers and potentially cause colony collapse. Other mites such as *Acarapis woodi* also infect honey bee colonies but rarely lead to colony collapse (Cepero *et al.*, 2015).

Cross-species transmission

While many of these parasites and pathogens have long been associated with one host species or genus, the coexistence of honey bees and bumblebees with wild bees, favoured by their domestication and commercial use for crop pollination, has increased the risk of horizontal transmission across pollinator species (Graystock *et al.*, 2014). Commercially reared *Bombus* spp. colonies are usually infected with parasites and pathogens (e.g. 77% of colonies with up to five different parasites; Graystock *et al.*, 2014) and their use in crop pollination has increased the spillover of parasites, such as *C. bombi* and *N. bombi*, into wild conspecifics and heterospecifics that forage close to the greenhouses where commercial colonies are used (Murray *et al.,* 2013; Cameron *et al.,* 2016). This is because commercial workers very often escape from greenhouses and forage outside, where they come into contact with wild flowers and pollinators (Whittington & Winston, 2004). A cross-genus infection from *Bombus* spp. to *A. mellifera* colonies has been reported

Table 1. Effects of parasites and pathogens on bee behaviour and cognition.

Parasite/pathogen	Transmission	Host	Effects on behaviour and cognition	References
Flies				
Apocephalus borealis	Direct infestation: parasitoids lay eggs in the host	Honey bees/bumblebees	Altered circadian rhythm; unusual night activity; disorientated flights due to light attraction	Core *et al.* (2012)
Conopid spp. *flies*	–	Bumblebees	Altered flower choices (prefer flowers that are easy to detect with facilitated access to pollen); altered circadian rhythm; stay outside the nest at night	Schmid-Hempel and Schmid-Hempel (1990)
			Hosts dig themselves into soil before being killed by the parasite	Müller (1994)
Nematode				
Sphaerularia bombi	Horizontal: *S. bombi* third-instar larvae come out of the bumblebee anus and penetrate new bumblebee queens during hibernation	Bumblebees	Spring queens look for hibernation sites instead of nests	Madel and Madel (1973)
Mites				
Varroa destructor	Horizontal: direct contact between hosts	Honey bees	Spend more time outside the colony	Kralj and Fuchs (2006)
			Impaired olfactory non-associative learning; reduced homing abilities	Kralj *et al.* (2007)
			Precocious onset of foraging; impaired thermoregulation	Downey *et al.* (2000)
Acarapis woodi	Horizontal: direct contact between hosts	Honey bees	Impaired flight (due to muscle reduction) increased food consumption	McMullan and Brown (2009)
Fungi				
Ascosphaera apis	Horizontal: ingestion of spores	Honey bees	?	–
Nosema apis	Horizontal: ingestion of spores (e.g. trophallaxis; cleaning tasks; contaminated surfaces), copulation (from drones to queens)	Honey bees	Perform more daily flights of shorter duration	Dosselli *et al.* (2016)
Nosema ceranae	Horizontal (see *N. apis*)	Honey bees/bumblebees	Impaired flight due to trehalose depletion (honey bees)	Dussaubat *et al.* (2013)
			Precocious onset of foraging (honey bees)	Goblirsch *et al.* (2013), Holt *et al.* (2013) and Natsopoulou *et al.* (2016)
			Reduced homing abilities (honey bees)	Wolf *et al.* (2014)
			Spend more time outside the nest (honey bees)	Kralj and Fuchs (2010)
			Reduced frequencies of trophallaxis (honey bees)	Naug and Gibbs (2009)
			Impaired olfactory associative learning	Piiroinen and Goulson (2016)

Table 1. Continued

Parasite/pathogen	Transmission	Host	Effects on behaviour and cognition	References
Nosema bombi	Horizontal (see *N. apis*); vertical: transovarian transmission from queen to larvae (only in *N. bombi*)	Bumblebees	?	–
Trypanosomatids				
Crithidia bombi	Horizontal: ingestion of the parasite	Bumblebees	Impaired foraging activity (visit fewer flowers, spend more time handling flowers and spend more time flying between flowers)	Otterstatter *et al.* (2005)
	Vertical: within colony between individuals of different castes (e.g. a worker bee infects a younger queen)		Impaired visual learning	Gegear *et al.* (2006) and Otterstatter *et al.* (2005)
Viruses				
Deformed wing virus (DWV)	Indirect infestation: vectorised by *V. destructor*	Honey bees/bumblebees	Perform flights of shorter duration and distance (honey bees)	Wells *et al.* (2016)
	Horizontal: faecal-oral, cannibalism; vertical: parent–offspring		Reduced homing abilities (honey bees)	Iqbal and Mueller (2007)
Israeli acute paralysis virus (IAPV)	Indirect infestation: vectorised by *V. destructor*; horizontal: oral ingestion	Honey bees	Reduced homing abilities	Li *et al.* (2013)
Kakugo virus (KV)	Horizontal: oral ingestion (e.g. trophallaxis)	Honey bees	Increased aggressiveness	Fujiyuki *et al.* (2004)

Question marks indicate that the effects are not known. Full references are available in the reference list.

for the neogregarine parasite of bumblebees *Apicystis bombi* (Apicomplexa: Neogregarinorida) (Plischuk *et al.*, 2011), yet the impact of this parasite on their new host species is still not well documented. Additionally, honey bee pathogens have been reported in bumblebees as well as in solitary bees and wasps (Ravoet *et al.*, 2014). Examples include *N. ceranae* (Fürst *et al.*, 2014; Arbulo *et al.*, 2015), or viruses like the DWV, the black queen cell virus (BQCV), the ABPV and the sacbrood bee virus (SBV) (Fürst *et al.*, 2014; McMahon *et al.*, 2015; Parmentier *et al.*, 2016). In contrast to social bees, which can rapidly replace infected and non-efficient foragers with new recruits in the colony, any impairment of the cognitive abilities of solitary bees that forage for their own brood may directly compromise the survival of the larvae, with dramatic consequences for local populations (Klein *et al.*, 2017).

How do parasites and pathogens affect bee behaviour and cognition?

Transition to foraging

While parasites and pathogens primarily impact the physiology of bees, there is growing evidence that behaviour and cognition can also be affected (see Table 1). In honey bees, division of labour is based on age, so that only middle-aged to old adults forage. The transition from in-hive activities (e.g. brood nursing) to foraging depends on a complex developmental program and a period of brain maturation to prepare them for the cognitive demands of foraging (Withers *et al.*, 1993; Fahrbach *et al.*, 1998). This behavioural change, which normally happens between 12 and 15 days after emergence from the pupae (Robinson & Vargo, 1997; Siegel *et al.*, 2013), is flexible and associated with a decrease of vitellogenin and an increase of juvenile hormone in the foraging bee (Guidugli *et al.*, 2005). A deficiency of food in the colony, or a reduced number of foragers, triggers a precocious onset of foraging in young bees (Toth *et al.*, 2005; Higes *et al.*, 2010). Parasites can also induce these behavioural modifications (Goblirsch *et al.*, 2013; Holt *et al.*, 2013; Natsopoulou *et al.*, 2014). For instance, colonies infected with the microsporidian *N. ceranae* experience significant losses of foragers that can be compensated with the precocious onset of foraging of young bees, regulated by hormone titres in the haemolymph (Antúnez *et al.*, 2009). Precocious foragers have poorer spatial cognitive abilities (Ushitani *et al.*, 2016), presumably because of insufficient brain maturation and foraging experience, resulting in a reduction in the stored food in the colony (Botías *et al.*, 2013; Lach *et al.*, 2015), which further promotes the recruitment of new precocious foragers. Through a snowball effect, when this situation is prolonged, the breaking down in the division of labour can lead to colony collapse (Khoury *et al.*, 2013; Perry *et al.*, 2015).

Foraging activity

Both parasites and pathogens can affect the foraging activity and flight ability of bees. Gegear *et al.* (2005) observed bumblebees foraging on artificial flowers in which they had to

land on the corolla and crawl inside a tube to gather the nectar. Despite training, bumblebees infected with the trypanosomatid *C. bombi* took longer to handle flowers (i.e. accessing and ingesting nectar) and rejected a greater proportion of flowers by landing on them and leaving without feeding than uninfected bees (Gegear *et al.*, 2005). When presented with blue and yellow flowers with equal rewards, infected bumblebees showed normal levels of flower constancy (the tendency of bees to forage on a single flower type), but much lower flower visitation rates compared with uninfected individuals, which could result in reduced foraging efficiency in natural conditions (Otterstatter *et al.*, 2005). Altogether these results suggest that parasites impaired both the motor behaviour of bees and their ability to evaluate flower rewarding value when making foraging decisions.

Likewise, honey bees infected with the microsporidia *Nosema* spp. have reduced foraging performances when compared with uninfected conspecifics. Parasitised foragers show an increased activity (Dussaubat *et al.*, 2013; Alaux *et al.*, 2014; Wells *et al.*, 2016), by performing more foraging trips of shorter duration with many stops between them (Dussaubat *et al.*, 2013; Wolf *et al.*, 2014; Dosselli *et al.*, 2016), and spending more time outside the colony (Kralj & Fuchs, 2010). In addition, honey bees infected with the DWV virus show reduced flight distances and durations when tested in a flight mill arm (Wells *et al.*, 2016). These changes in flight behaviour have been associated with the energetic stress caused by the parasites and pathogens that obtain resources from their hosts, who in turn lack nutrients to perform their tasks adequately. In the case of *N. ceranae*, infected honey bees present a reduction of the trehalose titres in the haemolymph, which alters their ability to produce ATP and fly (Mayack & Naug, 2010).

Learning and memory

Successful foraging requires bees to learn to locate and recognise flowers, to associate them with a food reward, and to navigate back to their nest location, based on visual, olfactory and spatial learning and memory (Menzel, 2012; Collett *et al.*, 2013; Giurfa, 2013).

Most research on olfactory cognition in bees has been conducted using the conditioning of the proboscis extension reflex (PER), an experimental paradigm where harnessed bees must learn to extend (or not) their proboscis in response to odour or gustatory stimuli (Giurfa & Sandoz, 2012). Although PER assays may not fully replicate the behavioural sequences and cognitive operations of bees foraging on natural flowers (Mujagic & Erber, 2009; Mujagic *et al.*, 2010), they provide a practical means to quantify learning and memory performances for specific cognitive tasks in controlled conditions. For example, in a simple habituation task (non-associative learning where the repeated presentation to a stimulus reduces the response of the individual) honey bees infested with the mite *V. destructor* ceased to respond to sugar stimulations of the antennae faster (i.e. habituate faster) than non-infested conspecifics (Kralj *et al.*, 2007). When tested in the reverse sensitisation task (where the repeated exposure to a stimulus amplifies the response of the individual), infested bees showed

a lower response to an odour stimulus following sugar stimulation (Kralj *et al.*, 2007), thereby suggesting that mites interfere with the synaptic transmissions enabling these two forms of non-associative learning. In a more complex absolute conditioning task (associative learning where the individual must associate an odour with a sugar reward), both honey bees and bumblebees fed with the microsporidian parasite *N. ceranae* showed a reduced proportion of responses to the stimulus odour after 10 pairings of the odour with a sugar reward when compared with non-parasitised conspecifics (Piiroinen & Goulson, 2016). The fact that parasitised and non-parasitised bees responded similarly to the sugar stimuli when presented alone indicates that their ability to associate the odour with a reward, and therefore not their motivation, was specifically impaired by the parasite (Piiroinen & Goulson, 2016).

Visuo-spatial learning, involved in flower choices and navigation, can also be impaired by parasites and pathogens. In an associative visual learning task, where bumblebees must learn to discriminate flowers on the basis of their colour, infected individuals were slower to learn and reached lower acquisition scores (e.g. made more choices in the flowers of wrong colour) than uninfected individuals (Gegear *et al.*, 2006). Navigation, based on spatial learning, may be impaired too. Honey bees infected with the microsporidian *N. ceranae* perform shorter learning flights (non-foraging flights during which bees acquire spatial memories of the nest's surroundings for future foraging attempts) and these flights cover reduced areas, indicating a lower tendency to explore (Wolf *et al.*, 2016). Infected foragers also have reduced homing abilities (Wolf *et al.*, 2014). Orientation impairment and lower homing rates have also been observed in honey bees infected with the viruses DWV (Iqbal & Mueller, 2007), IAPV (Li *et al.*, 2013), and with the mite *V. destructor* (Kralj *et al.*, 2007). Whether these changes of spatial behaviours are caused by cognitive impairments or energetic stress is an open question.

Social interactions

Parasites and pathogens can also affect social interactions that are vital to the organisation of the colony. A striking example is the reduction of trophallaxis (mouth-to-mouth food exchanges supporting nutrient flow within the colony) in honey bees infected with the microsporidian *N. ceranae*, due to an increased appetite among bees that are less prone to share food with others (Naug & Gibbs, 2009). Other pathogens, such as the Kakugo virus, have been suggested to trigger aggressiveness in their hosts. This picorna-like virus is found specifically in the brain of guard honey bees (*Apis mellifera L. Italian*) that defend the colony against predators (Fujiyuki *et al.*, 2004).

Motor behaviour

Some parasites affect the central nervous system to manipulate the locomotion behaviour of the host in order to favour their own spread and reproduction (Hughes *et al.*, 2012). Well-known examples are the fungus *Entomophthora* spp. which makes ants climb onto grass tips for a better dissemination of its spores

(Loos-Frank & Zimmermann, 1976) and the parasitoid wasp *Ampulex compressa* which injects a poison into the brain of cockroaches in order to drag the paralysed 'zombie' cockroach into its colony to feed its offspring (Gal & Libersat, 2010). Recent work indicates that honey bees are also subject to such behavioural manipulations (Core *et al.*, 2012). Individuals parasitised by the parasitoid fly *Apocephalus borealis* have an altered circadian rhythm, often leaving the colony during the night with elevated risks of being disoriented by artificial lights that attract them (Core *et al.*, 2012).

What are the main defence mechanisms of bees?

At the individual level

Bees have evolved various mechanisms to combat parasites and pathogens (see examples in Fig. 1). The first line of defence is the cuticle (exoskeleton of insects) which is both a mechanical and a biochemical barrier covered by antimicrobial compounds (Mackintosh *et al.*, 1995). As a second defence, insects have developed an innate immune system based on cellular and humoral responses (Schmid-Hempel, 2005; Tsakas & Marmaras, 2010). Cellular defence is primarily mediated by haemocytes and includes phagocytosis, nodulation or encapsulation of pathogens such as bacteria, protozoa or nematodes. Humoral defence is based on the secretion of antimicrobial peptides (e.g. defensin, abaecin or hymenoptaecin in honey bees; Antúnez *et al.*, 2009), the use of reactive oxygen intermediates as killing molecules (Vass & Nappi, 2001) and activation of enzymatic cascades that regulate melanisation (Gillespie *et al.*, 1997). As described earlier, this immune response is costly to the hosts and can reduce their life span (Moret & Schmid-Hempel, 2000) as well as impair their cognitive functions (Mallon *et al.*, 2003; Alghamdi *et al.*, 2008). For instance, bumblebees in which the immune system was stimulated non-pathogenically with lipopolysaccharides – a component of the Gram-negative bacterial cell – showed a reduced ability to associate an odour with a reward in an absolute PER conditioning assay (Mallon *et al.*, 2003) and learnt much more slowly to discriminate the colour of rewarding flowers in a free-flying differential conditioning task (Alghamdi *et al.*, 2008).

Bees also adopt behavioural strategies to avoid or combat infections by parasites and pathogens. For instance, when presented to flowers both contaminated and not contaminated with the parasite *C. bombi*, bumblebees visit uncontaminated flowers more frequently, suggesting that they are capable of discriminating between contaminated and uncontaminated nectars. This discrimination presumably arises through odour cues and prevents them from being infected and contaminating the colony (Fouks & Lattorff, 2011). Nutritional decisions also have substantial effects on the health of insects, and an appropriate intake of specific nutrients can be beneficial to combat infections (Ponton *et al.*, 2011; Di Pasquale *et al.*, 2013; Povey *et al.*, 2014). For instance, caterpillars challenged with a highly virulent nucleopolyhedrovirus tend to increase their intake of protein, a 'self-medication' behaviour that influences the constitutive immune function and augments the resistance to the pathogen attack (Lee *et al.*, 2006). Although there is no direct
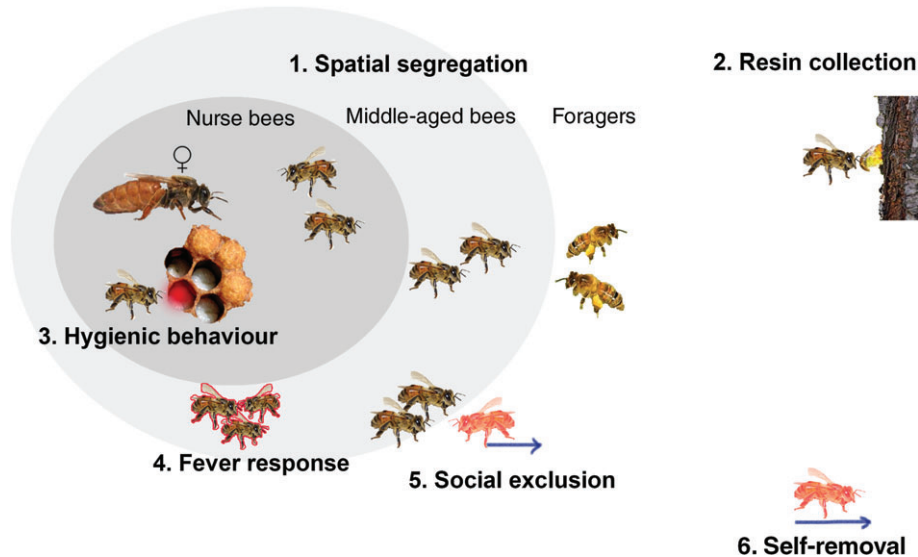
(a)   Individual defence

1. Physical barrier

Cuticle

2. Immune response

Cellular	Humoral
Phagocytosis	Antimicrobial peptides (AMPs)
Encapsulation	Reactive oxygen intermediates
Nodulation	Melanisation

(b)   Collective behaviour

1. Spatial segregation

Nurse bees   Middle-aged bees   Foragers

2. Resin collection

3. Hygienic behaviour

4. Fever response

5. Social exclusion

6. Self-removal

Fig. 1. Defence mechanism used by honey bees against parasites and pathogens. (a) At the individual level, the cuticle is a mechanical and biochemical barrier against infection. Once contaminated, bees develop an immune response. (b) At the collective level, bees have evolved a suite of 'social immunity' behaviours that reduce the exposure to or contact with parasites, including hygienic behaviours, spatial segregation, resin collection, social exclusion and self-removal of infected individuals (red bees) from the hive.

evidence of a change of dietary nutrient intake by infected bees, bumblebees infected with the trypanosomatid *C. bombi* prefer sucrose solutions containing alkaloids (e.g. nicotine) over pure sugar water (Baracchi *et al.*, 2015). Accordingly, in field conditions, infected bumblebees forage more on plants producing secondary metabolites (alkaloids, terpenoids, iridoid glycosides) that can reduce their parasitic infections (Richardson *et al.*, 2015, 2016).

At the collective level

In addition to individual defences, social bees have developed cooperative behaviours to combat infections (Fig. 1), a suite of strategies known as 'social immunity' (Cremer *et al.*, 2007). These behaviours primarily serve to reduce exposure to parasites and the rate of transmission within colonies.

To prevent infections, honey bees obtain antimicrobial substances from plant resins (e.g. *Populus* spp.) which they mix with wax into a paste (propolis) that is spread within the nest (Simone *et al.*, 2009; Simone-Finstrom & Spivak, 2010). The propolis is used for controlling infections by reducing pathogen loads, such as the bacterium *P. larvae* (Antúnez *et al.*, 2008; Kamel *et al.*, 2013) and the fungus *A. apis* (Simone-Finstrom & Spivak, 2012). Another key prophylactic strategy is spatial segregation, by which in-hive bees (e.g. nurses) have reduced physical contacts with the foragers, which have higher risks of being exposed to parasites and pathogens on flowers (Stroeymeyt *et al.*, 2014).

Once infected, adults detect and sacrifice infested brood in order to minimise the rate of transmission. These hygienic behaviours are the main defensive mechanisms of honey bees against the fungus *A. apis* (Gilliam *et al.*, 1988), the mite *V. destructor* (Evans & Spivak, 2010; Mondet *et al.*, 2015) and the bacterium *P. larvae* (Spivak & Reuter, 2001). To

prevent further contacts with parasites, honey bees also exclude parasitised adults from the colony (Baracchi *et al.*, 2012) or attack them (Waddington & Rothenbuhler, 1976).

It has been proposed that parasitised honey bees exhibit 'self-removal' behaviours by spontaneously leaving the nest to prevent the colony from further contamination (Rueppell *et al.*, 2010), as suggested by the fact that foragers spend more time outside the nest and have a lower homing rate when parasitised by the mite *V. destructor* (Kralj & Fuchs, 2006). Likewise, honey bees infected with the microsporidian *N. ceranae* perform longer flights and spend more time outside the colony (Dussaubat *et al.*, 2013; Alaux *et al.*, 2014). Whether these behaviours are the manifestation of adaptive responses of the host to prevent parasite transmission or a manipulative strategy by the parasite to favour its own transmission is still an open question. Although neither of the hypotheses can be ruled out definitively with current observations, the cognitive impairments observed in bees infected with *N. ceranae* (Kralj & Fuchs, 2010; Wolf *et al.*, 2014) and the associated changes in brain functions (McDonnell *et al.*, 2013) tend to support this hypothesis of host manipulation.

Social bees have also evolved cooperative behavioural strategies to mitigate infections by specific parasites (Cremer *et al.*, 2007). A striking example is the 'social fever' by which honey bees beat their wing muscles in coordination to increase the temperature within the hives to levels that are lethal for parasites but not for the bees (e.g. *A. apis*; Starks *et al.*, 2000).

Multiple mating (or polyandry) by which the queen mates with several males may also be a mechanism to reduce parasite loads in social bees (Baer & Schmid-Hempel, 1999; Palmer & Oldroyd, 2003). Polyandry increases the level of intra-colony genetic variability by generating separate patrilines (i.e. groups of bees who share the same father) with different susceptibilities to parasites and pathogens, potentially reducing their spread within the colony. For instance, honey bee colonies composed of more patrilines have lower prevalence of *A. apis* (Tarpy, 2003), *N. ceranae* (Desai & Currie, 2015) and *P. larvae* (Seeley & Tarpy, 2007). Likewise, the prevalence of the trypanosomatid *C. bombi* is reduced in genetically diversity colonies of experimentally inseminated bumblebee queens (Baer & Schmid-Hempel, 2003).

Conclusions and future directions

Parasites and pathogens are major stressors contributing to the widespread bee declines in most of the industrialised world (Goulson *et al.*, 2015; Klein *et al.,* 2017). We have described how some of these biological antagonists impact bee behaviour, cognition and foraging performance, ultimately compromising brood development and colony survival. Here we highlight some important directions for future research on bee-parasite ecology (see examples in Box 1).

BOX 1: Questions to be addressed in future research on bee-parasite interactions.

1 Are solitary bees similarly vulnerable to parasites and pathogens as social bees? Most studies on bee–parasite

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interactions have focused on honey bees and bumblebees, two historical models for experimental research on bees that are also of great economic importance (Menzel, 2012; Giurfa, 2013). Despite being an important first step, these bees are highly social (eusocial) and do not represent the wide diversity of social lifestyles observed in wild bees. Comparative research is urgently needed to assess the impact of parasites and pathogens on solitary bees as well, for which the costs of reduced foraging performances and the impact on pollination services are potentially much higher.

2 How do bees get contaminated? Are they attracted to cues produced by the parasites? Or to flowers that have had previous contact with parasitised bees? Can they communicate such information? Although there is knowledge about parasite and pathogen transmission among bees, little is known about how these biological antagonists are actually acquired in the environment and how they contaminate populations. Flowers are an important source of infection, harbouring a great variety of parasites and pathogens (Mcart *et al.*, 2014), and may play a central role in horizontal transmission between bees (Durrer & Schmid-Hempel, 1994).

3 Are cognitive abilities really impaired? If so, what cognitive functions are affected? What types of memories? Neuro-ethological studies using well-defined learning assays are needed to characterise the effects of parasites and pathogens on specific forms of learning and memory, for instance, using cognitive tasks of various complexities from non-associative learning (e.g. habituation, sensitisation; Menzel, 2012) to elemental associative learning (e.g. differential, absolute conditioning; Piiroinen & Goulson, 2016) and non-elemental associative learning (e.g. sameness/difference; Giurfa *et al.* (2001)). Such research will help us to better delineate the mode of action of parasites and pathogens on the nervous system, e.g. by locating impaired brain areas and neural circuits underpinning specific cognitive tasks (Devaud *et al.*, 2015).

4 Many insects combat parasites and pathogens by altering their nutrient intake to activate their immune system (Lee *et al.*, 2006; Riddell & Mallon, 2006; Povey *et al.*, 2014). Whether and how nutritional self-medication occurs in bees is an open question of considerable interest in assessing the resilience of populations. In social bees, this would require foragers to self-medicate through their feeding decisions, and also all the other colony members, including the larvae and the adults of different castes (nurses, queens, drones), whose nutritional needs can be altered due to their infection status (infected, uninfected) (Lihoreau *et al.*, 2014).

5 Do the gut microbiota affect the behaviour and cognition of bees? Internal microbes can have wide-ranging influence on insect behaviour (Wong *et al.*, 2015), e.g. by affecting mating preferences (Sharon *et al.*, 2010) or collective behaviours (Tan *et al.*, 2015). The gut of honey bees contains a distinctive and specialised microbiota that

can be experimentally manipulated (Kwong & Moran, 2016), thus holding considerable promise for studying behavioural microbiomics in these social insects.

6 For a complete understanding of host–parasite coevolution, it is required that adaptive host responses can be distinguished from manipulations by parasites or pathogens. Behavioural observations of the host alone are unlikely to provide such information. Neurogenomic profiling that provides an overview of the gene expression changes occurring in the bee brain in response to pathogens (McDonnell *et al.*, 2013), combined with functional analyses of changes in neural circuit performance, are needed to characterise precisely the causes of altered behaviour in infected bees. Studies of the parasite's performances under various host conditions can also explain the selection pressures exerted by hosts on parasites and help to interpret the behaviour of contaminated bees.

While it is becoming increasingly clear that many parasites and pathogens affect bee cognition, so far most of these observations only provide indirect or non-definitive evidence, for instance, through measures of the flight activity of bees (Dussaubat *et al.*, 2013; Alaux *et al.*, 2014), changes in their social interactions (Naug & Gibbs, 2009; Goblirsch *et al.*, 2013; Holt *et al.*, 2013) or reduced homing rates (Wolf *et al.*, 2014). In the latter case, alternative explanations to impaired spatial memories and disorientation, such as reduced flight abilities, increased energetic stresses or a loss of motivation to forage cannot be ruled out. Studies that actually compare learning and memory performance between infected and uninfected bees remain scant and use relatively simple cognitive tests (Kralj *et al.*, 2007; Charbonneau *et al.*, 2016; Piiroinen & Goulson, 2016), typically a differential associative learning task – where bees must learn to respond positively to a rewarded stimulus (CS+) and not to respond to an unrewarded stimulus (CS−) – based on the conditioning of the proboscis extension reflex (but see Gegear *et al.*, 2006). While this is an important first step, these assays are still far from encompassing the diversity of the cognitive problems bees must solve when foraging on natural flower patches (Giurfa, 2013). Do parasites and pathogens affect all sensory modalities (e.g. visual, olfactory, gustatory, tactile), all types of learning (non-associative, associative, elemental, non-elemental), and all types of memories (early short-term, mid-term, late short-term, and long-term memories)? What are the brain areas and the neural circuits involved? Are all bees equally impacted by these effects, and at all stages (e.g. young, old, workers, foragers)?

Advances in neuro-ethology and cognitive ecology now provide a wide range of behavioural assays in controlled laboratory or semi-field conditions to start answering these questions. In particular, new methodological approaches, such as high-throughput automated behavioural tracking using radio frequency identification (Henry *et al.*, 2012), barcode labels (Crall *et al.*, 2015), harmonic radars (Riley *et al.*, 2005) or computer vision (Lihoreau *et al.*, 2016) can be used to automatically quantify and compare the foraging behaviours of individually marked bees of known age, foraging experience and infection status as they learn to solve specific tasks in ecologically relevant conditions. Increasingly, these observations can be confronted with predictions of learning models (Reynolds *et al.*, 2013; Peng & Chittka, 2016) to understand how the cognitive processes are impacted by parasites and pathogens. Detailed data at the individual and collective levels also allow researchers to assess these impacts on colonies and populations (Khoury *et al.*, 2011; Becher *et al.*, 2014). Fast development of virtual reality assays, in which tethered bees walk on a locomotion compensator or fly to make foraging decisions in response to stimuli displayed on a screen, also hold considerable promise in characterising the effects of parasites and pathogens on behavioural responses to precisely manipulated stimuli and explore their neural underpinnings (Paulk *et al.*, 2014).

Ultimately, research into the effects of parasites and pathogens on the cognition of honey bees and bumblebees should be expanded to a wider range of pollinator species, starting with the large diversity of solitary bees whose general contribution to pollination is currently being re-evaluated (Garibaldi *et al.*, 2013). Broadening the scope of bee-parasite research is necessary to assess the impact of bee population declines on the global pollination services and to develop a more accurate evolutionary understanding of these complex interactions. As the evolutionary processes and dynamics differ between the hosts and their parasites and pathogens (Schmid-Hempel, 2011), understanding the selection pressures that shape phenotypic changes in the hosts as a result of their interactions with parasites will require a better integration of experimental and theoretical approaches from parasitology, epidemiology, animal behaviour and cognition into bee-parasite ecology research.

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