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Review Article

Honey bee survival mechanisms against the parasite *Varroa destructor*: a systematic review of phenotypic and genomic research efforts

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ABSTRACT

The ectoparasitic mite *Varroa destructor* is the most significant pathological threat to the western honey bee, *Apis mellifera*, leading to the death of most colonies if left untreated. An alternative approach to chemical treatments is to selectively enhance heritable honey bee traits of resistance or tolerance to the mite through breeding programs, or select for naturally surviving untreated colonies. We conducted a literature review of all studies documenting traits of *A. mellifera* populations either selectively bred or naturally selected for resistance and tolerance to mite parasitism. This allowed us to conduct an analysis of the diversity, distribution and importance of the traits in different honey bee populations that can survive *V. destructor* globally. In a second analysis, we investigated the genetic bases of these different phenotypes by comparing 'omics studies (genomics, transcriptomics, and proteomics) of *A. mellifera* resistance and tolerance to the parasite. Altogether, this review provides a detailed overview of the current state of the research projects and breeding efforts against the most devastating parasite of *A. mellifera*. By highlighting the most promising traits of *Varroa*-surviving bees and our current knowledge on their genetic bases, this work will help direct future research efforts and selection programs to control this pest. Additionally, by comparing the diverse populations of honey bees that exhibit those traits, this review highlights the consequences of anthropogenic and natural selection in the interactions between hosts and parasites.

1. Introduction: towards a stable host-parasite relationship between *Apis mellifera* and *varroa destructor*?

The ectoparasitic mite *Varroa destructor* (hereon *Varroa*) is indisputably the most significant pathological threat to the western honey bee, *Apis mellifera*, worldwide (Van Engelsdorp et al., 2008; Dietemann et al., 2012). In the wake of its global spread during the 1950–1990s, this parasite has severely impacted the management and profitability of beekeeping (Le Conte et al., 2010; Carreck and Neumann, 2010). The mite is completely dependent on the honey bee colony, with a reproductive cycle synchronized to host pupa development inside brood cells while feeding on brood and adult bee haemolymph and fat body tissue (Ramsey et al., 2019). However, the most devastating impact of the mite is that it is a biological vector for honey bee viruses (Martin et al., 2012; Mondet et al., 2014; Wilfert et al., 2016). In the absence of the mite, these viruses persist in colonies as covert infections. However, the opportunistic nature of these viruses, together with exponential mite population growth, quickly results in the development of lethal virus epidemics that typically kill a colony within 2–3 years (Amdam et al., 2004).

Colonies of the mite's natural host, the eastern honey bee (*Apis cerana*), are generally not threatened by *V. destructor* due to a stable host-parasite relationship that has been established over a long evolutionary scale (Oldroyd, 1999). Such a relationship is distinguishably missing with the new host, *A. mellifera*, which acquired the mite after colonies were transported into northeastern Asia (Kulikov, 1965; Crane, 1968). *Apis cerana* has a variety of defence mechanisms that limit the mite's population growth (Peng et al., 1987; Boecking et al., 1993; Page et al., 2016;

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Lin et al., 2018). While *A. mellifera* shares some of these defence mechanisms, they are far less pronounced (Fries et al., 1996). As a result, varroa mites are able to maximize reproductive opportunities, which ultimately results in exponential mite population growth to lethal levels (Martin et al., 1998; Calis et al., 1999).

Initial responses of researchers and beekeepers to varroa focused heavily on chemical treatments to control the mite. Today, most managed *A. mellifera* colonies depend on mite control treatments to survive (Rosenkranz et al., 2010). These chemical treatments can actually harm honey bees (Johnson et al., 2009; Locke et al., 2012a), leave residues in hive products (Johnson et al., 2013) and can become ineffective as *V. destructor* populations can swiftly become resistant (Milani, 1999; Beaurepaire et al., 2017). Additionally, mite control treatments also remove the selective pressure of natural mite infestation, preventing coevolutionary processes towards a stable host-parasite relationship (Neumann and Blacquière, 2016).

An alternative approach to reduce the dependency on chemical treatments has been to selectively enhance heritable resistance or tolerance to the mite through breeding programs (Büchler et al., 2010; Rinderer et al., 2010; see Fig. 1A for a timeline of breeding programs). This approach has yielded some success but it is tedious work and often based on genetically complex behaviour that is difficult to phenotype (Dekkers and Hospital, 2002; Beaurepaire et al., 2019a). However, recent advances in biotechnology can help facilitate selection. For instance, causative genes and proteins associated with resistance or tolerance can be developed as marker-assisted selection (MAS) tools for improving breeding stock at a large scale (Grozinger and Robinson, 2015; Guarna et al., 2017).

In addition to selective breeding, natural selection has yielded honey bee populations in Europe, North America, South America and Africa that survive varroa without parasite management (Locke, 2016; Fig. 1A). The underlying mechanisms are not all well understood, and seem to vary between different naturally selected populations despite experiencing similar selection pressures (Locke et al., 2012b; Oddie et al., 2018a).

In this review, we conducted a literature survey of all studies documenting phenotypic features (i.e., traits) of *A. mellifera* populations either selectively bred or naturally selected for resistance and tolerance towards varroa. We evaluated 153 studies in total, published between 1984 and 2019 (Fig. 1B). For each study we reviewed, we asked the following questions: (i) Were the bees selectively bred or naturally selected? (ii) What are the investigated traits and are they similar across studies and populations? (iii) Are there common molecular pathways involved in different studies and populations? Our aim was to systematically evaluate the most promising traits of varroa-surviving bees to help direct future research efforts and selective breeding programs.

2. Literature review of studies documenting resistance / tolerance / survival to *varroa*

2.1. History of research on honey bee resistance and tolerance traits

Since *V. destructor* has only shared a short co-evolutionary history with *A. mellifera*, very little was known about the relationship between the novel host and the parasite. Investigations were therefore conducted on *A. cerana*, the original host, to decipher the mechanisms underlying the balanced host-parasite relationship in that bee species. Pioneering studies identified several mite resistance traits such as grooming behaviour (Peng et al., 1987), reduction of mite fertility and varroa-sensitive hygiene behaviour (VSH) (Rath and Drescher, 1990; Boot et al., 1999). Other passive characteristics such as a short post-capping duration for brood cells also contribute to host-parasite equilibrium in the native host. For example, the post-capping periods for worker and drone brood of *A. cerana* are approximately 11 and 14 days, respectively (Rosenkranz and Engels, 1994). Since a foundress (mother) mite lays female eggs once every 30 h, and the daughter mite needs to be completely mature to survive when the host bee emerges (Martin, 1994), fewer daughter mites can be produced in an *A. cerana* brood compared with *A. mellifera*.

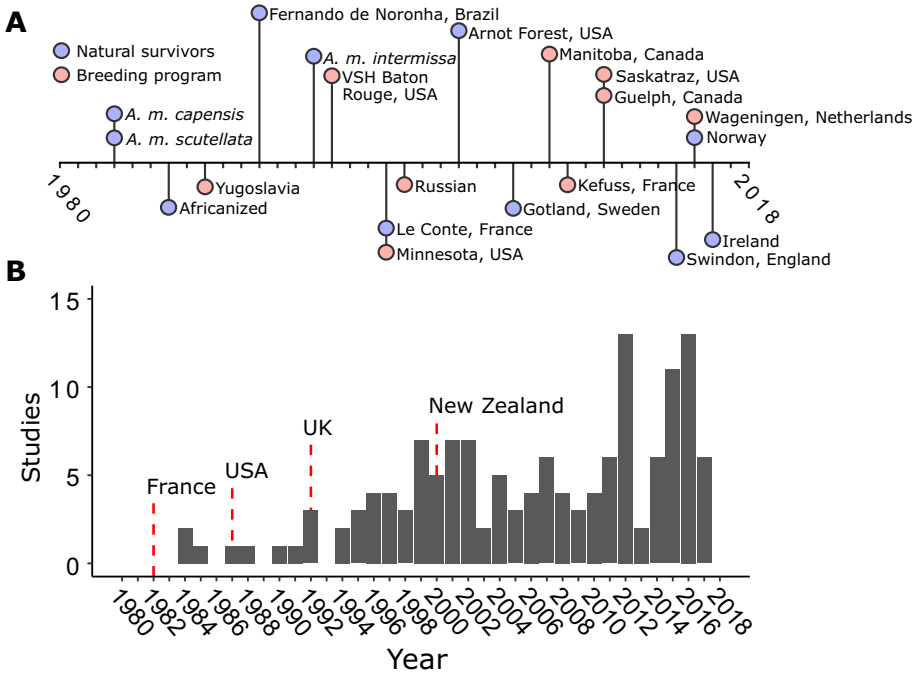

Fig. 1. Publication record on the topic of *Varroa* mite survival. (A) Study initiation on naturally surviving populations (purple) and breeding program populations (pink). (B) Number of studies published per year. Red lines indicate dates of *Varroa* introduction in different countries. *Varroa destructor* was first found on *Apis mellifera* (USSR) in 1949, in Europe in 1967 (Bulgaria), in South America in 1971 (Paraguay) and in Africa in 1975 (Tunisia).

In contrast to *A. cerana*, the development of *A. mellifera* worker brood typically takes 1 day longer; therefore, more female mites can reach maturity. However, two African Western honey bee subspecies – *Apis mellifera capensis* and *Apis mellifera scutellata* – have significantly shorter worker brood post-capping stages than *Apis mellifera carnica* (a European subspecies) (Moritz, 1985). These subspecies are better able to resist varroa mites (Moretto et al., 1995; Rosenkranz, 1999), providing a further link between post-capping duration and varroa resistance (Moritz, 1985).

Selectively breeding honey bees with improved varroa resistance would be beneficial for beekeeping and reduce dependence on acaricides. To this end, heritability analyses have been conducted to quantify the selection potential of traits that limit varroa population growth and efforts have been made to select resistant bees on this basis (Moritz, 1985; Le Conte et al., 1994; Harbo and Harris, 1999a; Boecking et al., 2000; Stanimirovic et al., 2008, 2010).

Another selection approach has been to leave colonies untreated, and to select the survivors for breeding. This risky approach, also called the "Bond Test", has been successfully developed in France (by J. Kefuss, France) and in Sweden (by I. Fries, SLU, Sweden). In some cases, no human interference was necessary, and colonies developed natural resistance or tolerance to the mite without human intervention. For example, two small populations in western and southeastern France exist as feral colonies which repopulated the area after they had been destroyed when varroa mites first invaded (Le Conte et al., 2007). Similar colonies have also been identified in the Arnot Forest in the northeastern USA (Seeley, 2007). However, the largest honey bee population that survives naturally with the mite are the Africanized bees in South America, Central America, and the southern United States (Rosenkranz, 1999). Naturally varroa-surviving populations may not be suitable for large-scale commercial beekeeping owing to undesirable beekeeping characteristics such as frequent swarming or low productivity, but they are precious for genetic diversity and biodiversity, as they can contribute to pollination in wild areas where managed honey bee colonies are less prevalent. Moreover, these colonies are good models for studying the mechanisms of a stable host-parasite relationship and thus to characterize the specific phenotypes that could be selected to support beekeeping.

2.2. VSH: a case study to illustrate the temporal changes in our understanding of mechanisms for survival

Behavioural traits are an important part of honey bees' social immunity repertoire (Cremer et al., 2007). However, these traits are complex, ranging from performances of single bees to groups of individuals performing different stages of what is considered a single activity (Spötter et al., 2016), making it difficult to do comparative research on expression of the behaviour and effective selection in breeding programs (Fonio et al., 2012; Bergman and Beehner, 2015).

A case study of research directed at this problem comes from a U.S. Department of Agriculture (USDA) laboratory in Louisiana, USA. In 1995, researchers identified colonies with negative mite population growth (MPG; Table 1) during a short (10 week) test under highly controlled experimental conditions (Harbo and Hoopingarner, 1997). The factor most strongly associated with low MPG was a high frequency of non-reproducing varroa foundresses. This characteristic had been observed previously in mite-resistant populations of *A. mellifera* in Uruguay (Ruttner et al., 1984), Tunisia (Ritter, 1990), and Argentina (Eguaras et al., 1995). The trait associated with high non-reproduction in the U. S. bees came to be called suppressed mite reproduction (SMR) (Harbo and Harris, 1999a). SMR was determined to be heritable (Harbo and Harris, 1999b) and became the focus of selective breeding for resistance (Harbo and Harris, 2003). While it is reasonable to expect that the brood of SMR bees somehow caused the poor mite reproduction (Milani et al., 2004), it was later discovered that the selected bees expressed high levels of the trait of hygiene (HYG). Thus, an effect of the adult bees, not the brood, was the major driver of high non-reproduction in mites (Harbo and Harris, 2005). Surprisingly, SMR bees removed more mite-infested pupae than bees which had been selectively bred for hygienic removal of freeze-killed brood (Spivak, 1996; Ibrahim and Spivak, 2006). Moreover, the hygienic activity of SMR bees appeared to be biased toward pupae parasitized by reproducing mites rather than non-reproducing mites (Harbo and Harris, 2005; Ibrahim and Spivak, 2006). This largely explained the higher proportion of non-reproducing mites in the SMR colonies, relative to unselected colonies. The SMR trait was thus renamed varroa sensitive hygiene (VSH) to emphasize the regulatory behavioural mechanism that governs high mite resistance (Harris, 2007).

Despite changes in understanding about the main mechanisms of mite resistance, selection for VSH has almost always been based

Table 1
Definitions of the terminology related to honey bee survival with *Varroa* infestations.

Terms	Definitions
Host tolerance	Ability of a host (honey bee colony or individual bee) to reduce the impairment caused by the parasite
Host resistance	Ability of a host (honey bee colony or individual bee) to reduce the reproductive success of the parasite so that the infestation stays below a damaging level
Honey bee trait	An observable structural or functional feature of honey bees or honey bee colonies
Regulatory trait	A heritable structural, physiological or behavioural trait that confers resistance or tolerance to the host against a parasite
Mite fertility	Natural ability of a foundress mite to produce at least one egg
Mite fecundity	Abundance of eggs laid by a given foundress, or the reproductive rate measured by the number of eggs produced
Mite population growth	The change in the number of mites in a population over a specified time. No (or low) population growth is the basis of host resistance
Mite non-reproduction (MNR)	Failure of a foundress mite to produce at least one adult, mated female that will enter the colony's mite population when the developing bee emerges from the cell as an adult bee. A foundress mite will not be successful at reproduction if she does not lay any eggs (infertile), lays only one egg, produces no male offspring or begins laying her eggs too late in relation to the pupal development
Suppressed mite reproduction (SMR)	Redefined as only cases of mite non-reproduction that are regulated by traits expressed by the brood
Hygienic behaviour (HYG)	Behavioural sequence consisting of the targeting, opening and removal of diseased, injured, parasitized, or dead brood by worker bees. This trait is usually assessed using the freeze-killed brood (FKB) or pin-killed assays
Varroa-sensitive hygiene (VSH)	Form of hygienic behaviour that specifically targets and removes brood infested by *Varroa* mites. This trait is assessed through assays that measure the removal of *Varroa*-parasitized brood
Recapping	Behavioural sequence consisting of the targeting, opening and then recapping of brood cells, leading to the potential disruption of mite reproduction. More research is needed to confirm that this trait is totally distinct from VSH
Grooming	Behaviour consisting of the removal of *Varroa* from adult bees, either by a bee infested by a mite itself (autogrooming) or by a bee cleaning another bee (allogrooming)
Mite virulence	Ability of the parasite (mite) to inflict harm on its host

on the frequency of non-reproducing mites in a colony (although there was some selection by quantifying removal of mite-infested brood). Traits other than HYG (e.g., disruptive semiochemical production by the brood or physiological modifications of the parasitized pupae) could contribute to high mite non-reproduction, and such mechanisms should be retained during selection. Indeed, compounds released by infested brood can compromise varroa reproduction and result in increased MNR, independently from an action of the adult bees (Nazzi and Milani, 1996, Milani et al., 2004). Recent evidence also suggests that there may be a brood effect contributing to HYG and VSH (Wagoner et al., 2018, 2019) although such an effect has been difficult to stabilize through breeding (Villa et al., 2016). We propose here to rename the phenotypic feature of high mite non-reproduction as MNR because it may be derived from both VSH activity and an effect of bee brood (Table 1). We recommend reserving the term SMR for non-reproduction of mites that is induced solely by the brood.

2.3. Qualitative and quantitative analyses of traits related to *A. mellifera* survival with Varroa

We conducted a literature survey to understand the traits linked to the ability of honey bees to survive varroa after natural selection or targeted breeding. Publications were identified through a comprehensive search on Web of Science (last updated in March 2019), using the search string: 'varroa AND [surv* OR resist* OR tol*]'. A filter was applied to include only studies performed on colonies that had been untreated for varroa for a minimum of 2 years, to meet the definition of 'surviving' (Mondet and Conte, 2014). We identified 78 studies, corresponding to 20 different surviving populations worldwide (Table 2, Fig. 1A).

Among the different *A. mellifera* populations, Africanized honey bees are the most studied, with 19 publications focusing on this group, mainly in Brazil and Mexico (Fig. 2). The second and third most studied populations are honey bees specifically bred by the USDA for the SMR/MNR and VSH traits (VSH Baton Rouge), or for low MPG (Russian bees from Primorsky), with 10 and eight publications, respectively. The remaining populations have been reported in one to six publications each. This highlights the heterogeneity of the different populations which have been investigated. Individual studies were performed on between one and 100 colonies each, with a mean of 12 colonies per study and population (+/− 10.6), leaving doubts on the importance of some traits in specific populations (Table 2). The observations were made between 1983 and 2017, showing that the mechanistic study of honey bee survival with *Varroa* infestations has an historic precedence, which started shortly after the introduction of the mites in western Europe (Ruttner and Ritter, 1980) (Fig. 1B).

Many phenotypes have been studied in the surviving populations of *A. mellifera*, ranging from individual bee to colony phenotypes (Tables 2 and 3). Varroa population dynamics resulting in reduced MPG, as measured by brood infestation, phoretic infestation, or natural mite mortality, has been investigated in approximately 40% of the studies (31 publications). Low MPG has been confirmed in 25 out of these 31 studies, comprising 95% of the studied populations (19 out of 20). This confirms that in most surviving populations, the ability of colonies to maintain mite numbers below a damaging level is a central feature. Varroa resistance mechanisms, rather than tolerance mechanisms, are thus more likely to explain honey bee survival with *Varroa* infestations.

We distinguished 15 putative mechanisms that were investigated and further confirmed as primary regulatory traits that are involved in the survival of honey bee colonies with *Varroa* infestations (Table 3). The literature review approach for comparative phenotypic analyses should be interpreted with care since several factors can influence publication frequency beyond the importance of a given trait in a population. When considering all *A. mellifera* populations, grooming appears as the most studied trait (18 studies), followed by VSH and MNR (17 studies each) (Fig. 3). MNR was the trait confirmed in most studies (14 studies, seven populations), followed by VSH (10 studies, five populations) and grooming (10 studies, five populations).

Interestingly, beyond MNR and VSH, recapping is also frequently verified to have an important role; it has been identified in seven different studies documenting eight different populations (Fig. 3). By recapping cells, it is believed that honey bees can disrupt the mite's reproductive cycle and cause lower fecundity of the foundress (Oddie et al., 2019). Although researchers began investigating this trait in the mid-1990s (Aumeier et al., 2000), we notice a re-emerging interest in recapping in the recent years. The relative contribution of this trait to varroa resistance is still debated and needs further investigation (Van Alphen and Fernhout, 2019).

There has been some disagreement in the literature over the contributing role of HYG, as selected using the freeze-killed brood or pin-test assays (Leclercq et al., 2018), for varroa resistance. HYG has been extensively tested in surviving populations, with eight studies investigating it and one confirming its potential involvement in honey bee survival with *Varroa* infestations (Table 2). Confusion exists around the concepts of VSH and HYG, and their respective associated assays. The behavioural motor sequence is identical in both cases: upon detection of a stimulus, workers uncap and remove the targeted cell contents. What remains unclear, however, is whether the detection step occurs through identical stimuli. Evidence suggests that it may not be the case (Nazzi et al., 2004; Mondet et al., 2016; McAfee et al., 2018; Wagoner et al., 2019). VSH is unarguably a specific form of HYG, but the freeze-killed brood or pin-test methods used to score HYG often fail to identify colonies that display VSH. Selectively bred VSH colonies (VSH BR population) adeptly remove freeze-killed brood, but bees bred for HYG using the freeze-killed brood assay (MIN population) remove low proportions of varroa-infested brood (Danka et al., 2013). Some studies confirmed that colonies bred for HYG displayed lower mite levels than control colonies (Spivak, 1996; Spivak and Reuter 1998; Ibrahim and Spivak, 2006, 2007), but since these studies were not performed on colonies untreated for at least 2 years, they were not included in the literature review. The sole use of the freeze-killed brood or pin-test assays does not appear to be sufficient to select for varroa resistance. Other assays that more directly quantify the removal of mite-infested brood are better tools to select for varroa resistance.

Excluding Africanized bees, *A. m. scutellata*, *A. m. capensis* and *A. m. intermissa* from the literature review, we found that the most frequently confirmed traits are MNR (11 studies, five populations), VSH (eight studies, four populations) and recapping (seven studies, six populations), with fewer studies confirming grooming (three studies and three populations, Table 3).

These comparative phenotypic analyses highlight the diversity of traits that appear to play roles in different surviving populations; no universal mechanism for survival emerged from this literature survey. In most cases, survival of both naturally and artificially selected populations is due to the expression of several traits that appear to collectively confer resilience to varroa infestation (Fig. 3). This seems true for the naturally selected populations as well as for the populations specifically bred for one or several traits. For the three best-documented naturally selected populations (Arnot Forest – USA, Gotland – Sweden, Le Conte – France), four to six traits seem to enhance varroa-survival abilities of bee colonies, highlighting the complexity of maintaining a stable host-parasite equilibrium. Interestingly, in populations selected

Table 2
Summary of the honey bee surviving populations highlighted by the literature review. For each documented population, the nature of the selection is indicated (Nat. surv, natural survival; Breed. Prog, breeding program). Mite population growth (MPG, indicated in italics) represents a downstream result of traits.

Population	Country	No. studies	Selection	Selection criteria	Years no treat	No. investigated traits	Investigated traits	No. verified traits	Verified traits	No. colonies	Study years	Refs
Africanized	Brazil, Mexico Puerto Rico	19	Nat. surv.	-	No treat	13	Grooming, VSH, recapping, brood susceptibility, mite fertility, cell size, HYG, mite fecundity, aggressiveness, MNR, mite haplotype, brood attractiveness, *MPG*	8	Grooming, VSH, mite infertility, cell size, MNR, mite haplotype, mite fecundity, *MPG*	3–29	1986–2010	Rosenkranz and Engels (1994), Message and Goncalves (1995), Aumeier et al. (1996, 2000, 2002), Corrêa-Marques and De Jong (1998), Guzman-Novoa et al. (1999, 2012), Medina and Martin (1999), Guerra (2000), Aumeier (2001), Vandame et al. (2002), Garrido et al. (2003), Mondragon et al. (2005, 2006), Carneiro et al. (2007), Strapazzon et al. (2009), Pinto et al. (2012), Rivera-Marchand et al. (2012)
Arnot Forest	USA	5	Nat. surv.	-	No treat	5	Small colonies, swarming, genetic isolation, high queen polyandry, *MPG*	3	Small hives, swarming, genetic isolation, *MPG*	6–23	2002–2016	Seeley (2007, 2017), Seeley et al. (2015), Tarpy et al. (2015), Loftus et al. (2016)
Capensis	South Af.	2	Nat. surv.	-	No treat	2	Post capping duration, *MPG*	2	Short post capping stage, *MPG*	5	1983–1988	Moritz et al. (1984, 1990)
Fernando de Noronha	Brazil	2	Nat. surv.	-	2 -15	3	Grooming, mite fertility, *MPG*	1	*MPG*	12–20	1991–1997	de Jong and Soares (1997), Corrêa-Marques et al. (2002)
Gotland	Sweden	6	Nat. surv.	-	6-20	8	Swarming, grooming, HYG, colony size, MNR, virus tolerance and resistance, recapping, *MPG*	6	Colony size, MNR, virus tolerance and resistance, recapping, *MPG*	4–23	2005–2016	Fries et al. (2006), Fries and Bommarco (2007), Locke et al. (2012a,b, 2014), Oddie et al. (2018a,b)
Guelph	Canada	1	Breed. prog.	MPG	1	2	Grooming, *MPG*	1	Grooming, *MPG*	13	2010	Guzmán-Novoa et al. (2012)
Intermissa	Algeria	1	Nat. surv.	-	2-9	1	*MPG*	1	*MPG*	9	1994–2002	Kefuss et al. (2004)
Ireland	Ireland	1	Nat. surv.	-	6	3	Grooming, DWV tolerance/ resistance, *MPG*	1	*MPG*	5	2016	McMullan (2018)
Kefuss	France	1	Breed. prog.	Survival	11	2	HYG, *MPG*	1	*MPG*	~100	2008	Kefuss et al. (2015)
Le Conte	France	6	Nat. surv.	-	2–13	7	Swarming, MNR, grooming, VSH, recapping, propolis, *MPG*	6	Swarming, MNR, VSH, recapping, propolis, *MPG*	2–21	1998–2016	Martin et al. (2001), Le Conte et al. (2007), Navajas et al. (2008), Locke et al. (2012), Popova et al. (2014), Oddie et al. (2018a,b)
Manitoba	Canada	1	Breed. prog.	Survival/ MPG	12	2	Grooming, *MPG*	2	Grooming, *MPG*	12	2007	Bahreini and Currie (2015)
Minnesota (MIN)	USA	3	Breed. prog.	HYG	2–12	4	VSH, MNR, recapping, *MPG*	3	VSH, recapping, *MPG*	3–63	1998–2011	Spivak and Reuter (2001), Ibrahim and Spivak (2006), Ibrahim et al. (2007)
Norway	Norway	3	Nat. surv.	-	17–19	6	MNR, grooming, VSH, recapping, post-capping duration, *MPG*	4	MNR, recapping, post-capping duration, *MPG*	5	2015–2017	Oddie et al. (2017, 2018a,b, 2019)
Russian	USA	8	Breed. prog.	MPG	11–15	6	MNR, grooming, DWV tolerance/resistance, VSH, recapping, *MPG*	6	MNR, grooming, DWV tolerance/ resistance, VSH,	4–32	1999–2013	Rinderer et al. (1999, 2001), Harris and Rinderer (2004), De Guzman et al. (2008), Guzmán-Novoa et al. (2012), Kirrane et al. (2015, 2018), Khongphinitbunjong et al. (2016)

(*continued on next page*)

Table 2 (*continued*)

Population	Country	No. studies	Selection	Selection criteria	Years no treat	No. investigated traits	Investigated traits	No. verified traits	Verified traits	No. colonies	Study years	Refs
									recapping, *MPG*			
Saskatraz	USA	1	Nat. surv.	-	11	3	Brood susceptibility, pathogen prevalence, *MPG*	3	Brood susceptibility, pathogen prevalence, *MPG*	1	2010	Robertson et al. (2014)
Scutellata	South Af.	5	Nat. surv.	-	No treat	6	VSH, post-capping duration, MNR, pathogen prevalence, brood susceptibility, *MPG*	4	Post-capping duration, MNR, pathogen prevalence, *MPG*	5–20	1983–2018	Moritz (1985), Strauss et al. (2013, 2016), Cheruiyot et al. (2018), Nganso et al. (2018)
Swindon	UK	1	Nat. surv.	-	18	1	DWV tolerance	1	DWV tolerance	3	2014	Mordecai et al. (2016)
VSH BR	USA	11	Breed. prog.	SMR/VSH/ MPG	5–17	8	HYG, VSH, MNR, recapping, grooming, DWV tolerance/ resistance, *MPG*	6	HYG, VSH, MNR, recapping, DWV tolerance/ resistance, *MPG*	1–43	1995–2016	Harbo and Hoopingarner (1997), Harbo and Harris (2005), Ibrahim et al. (2006, 2007), Harris et al. (2010, 2012), Le Conte et al. (2011), Tsuruda et al. (2012), Danka et al. (2013, 2016)
Wageningen	Netherlands	2	Breed. prog.	Survival	7–8	3	Grooming, VSH, MNR	1	VSH	5	2015–2016	Kruitwagen et al. (2017), Panziera et al. (2017)
Yugoslavia	Yugoslavia	1	Breed. prog.	Survival	4	1	*MPG*	1	*MPG*	9	1988	Kulinčević et al. (1992)

Years no treat, number of years colonies have been untreated against *Varroa*; VSH BR, Baton Rouge (USA) honey bee population bred for the VSH trait, HYG, hygienic behaviour; MNR, mite non reproduction; VSH, *Varroa*-sensitive hygiene; DWV, deformed wing virus; Af, Africa.

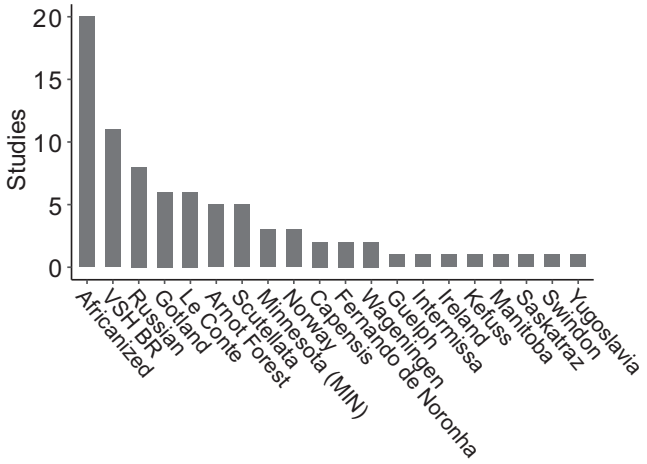

Fig. 2. Number of studies published for naturally *Varroa*-surviving populations and breeding program populations of honey bees. Africanized, Africanized honey bees (Brazil and Mexico); VSH BR, Baton Rouge VSH (*Varroa*-sensitive hygiene bred population (USA); Russian, Baton Rouge Russian bred population (USA); Gotland, Gotland Island surviving population (Sweden); Le Conte, Sarthe and Avignon surviving populations (France); Arnot Forest, Arnot Forest surviving populations (USA); Scutellata, *Apis mellifera scutellata* honey bees (South Africa); Minnesota, Minnesota hygienic bred population (USA); Norway, Norway surviving population; Capensis, *Apis mellifera capensis* honey bees (South Africa); F de Noronha, Fernando de Noronha surviving population (Brazil); Wageningen, Wageningen surviving populations (Netherlands); Guelph, Guelph breeding program (Canada); Intermissa, *Apis mellifera intermissa* surviving population (Algeria); Ireland, surviving population (Ireland); Kefuss, Kefuss surviving population (France); Manitoba, Manitoba breeding program (Canada); Saskatraz, Saskatraz surviving population (USA); Swindon, Swindon surviving population (UK); Yugoslavia, Yugoslavia breeding program (Yugoslavia).

Table 3
Summary of the regulatory traits governing honey bee survival with *Varroa* infestation, and associated proximate results.

Mechanism	Regulatory trait	Proximate result(s)
Resistance	*Adult bees*	
	Grooming	Phoretic infestation ↓
	VSH	MNR ↑; Brood infestation ↓
	Recapping	MNR ↑; Fecundity ↓
	HYG	Brood infestation ↓
	Cell size	Fecundity ↓
	Brood	
	"SMR" (as a true brood-based effect)	MNR ↑; Fecundity ↓
	Brood attractiveness	Phoretic/brood infestation ratio ↑
	Post-capping duration	Fecundity ↓
	High brood susceptibility	MNR ↑; Mortality ↑
	Colony	
	Swarming	Phoretic infestation ↓
	Small colonies	Brood infestation ↓
	Mites	
	Mite haplotype	MNR ↑; Fecundity ↓; Mortality ↑
Tolerance	Pathogen tolerance (virus, nosema, chalkbrood)	Better colony survival with high mite loads
	Self-medication/Propolis	Better colony survival with high mite loads
	Low brood susceptibility	Better colony survival with high mite loads

VSH, *Varroa*-sensitive hygiene; SMR, suppressed mite reproduction; MNR, mite non reproduction; HYG, hygienic behaviour.

and bred for specific phenotypes, several traits are also frequently found. The two best examples are the VSH Baton Rouge population (selected for low MPG, MNR and VSH) and the Russian population (selected for low MPG). Five and six traits were verified for each of

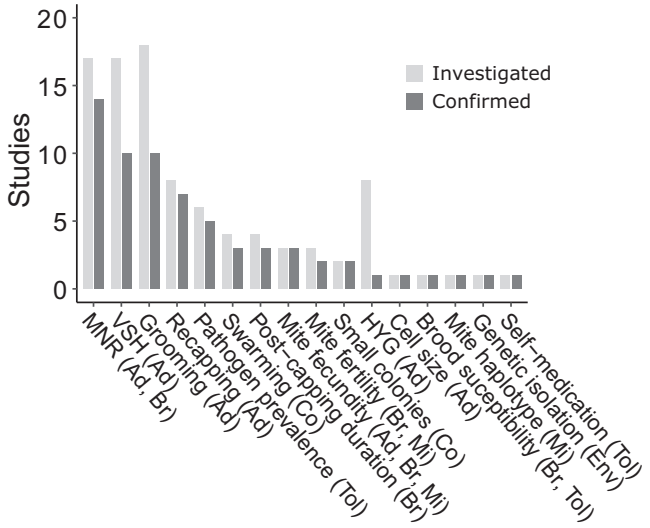

Fig. 3. Phenotypic features of honey bee populations that survive *Varroa* infestations. Number of studies investigating (light grey) or confirming (dark grey) each trait potentially involved in honey bee survival with *Varroa* infestations. Resistance traits can be attributed to an action from adult bees (Ad), the brood (Br), the colony (Co), mites (Mi) or environmental factors (Env). Tol, tolerance trait. MNR, Mite non reproduction; VSH, *Varroa*-sensitive hygiene, HYG, hygienic behaviour.

these two populations, respectively (Table 2). However, for the VSH Baton Rouge population, VSH is the primary trait that arose through selecting for high MNR, while the other traits are thought to contribute much less to mite resistance. Nevertheless, the repeated co-occurrence of traits in honey bee colonies with enhanced survival with *Varroa* infestations suggests that some of these traits might be related and regulated by common pathways and/or environmental parameters.

We urge researchers to distinguish the true regulatory mechanisms that are at the basis of bee survival with *Varroa* infestations (and which are at least partly genetically regulated) from the downstream results of those traits (Table 3, Fig. 4). Among the documented traits, five adult bee features (VSH, grooming, recapping, HYG, cell size), four brood features (MNR, post-capping duration, attractiveness, social apoptosis through high brood susceptibility), two colony features (swarming, colony size), one mite feature (virulence), and three tolerance features (tolerance of pathogens, self-medication through propolis, low brood susceptibility) stand as regulatory traits. On the other hand, parameters such as mite population dynamics, MNR and MPG appear as downstream consequences. Environmental conditions (such as climate and nutrition) and beekeeping practises can also actively contribute to honey bee survival with *Varroa* infestations (Fig. 4). Some of these traits have been verified by only a few studies or in studies performed mainly on colonies which had been treated against varroa, and thus need confirmation.

3. Review of studies using 'omics techniques to identify candidate genes for resistance, tolerance and survival

3.1. How can 'omics help us select for colony survival?

'Omics tools have helped identify molecular markers to support selective breeding and to understand the molecular mechanisms underlying varroa resistance, tolerance and survival traits. Genomics is used to identify DNA fragments (single nucleotide polymorphisms (SNPs) or quantitative trait loci (QTLs)), transcriptomics quantifies RNA expression of candidate causal genes, and proteomics quantifies protein abundance. Theoretically, any of these

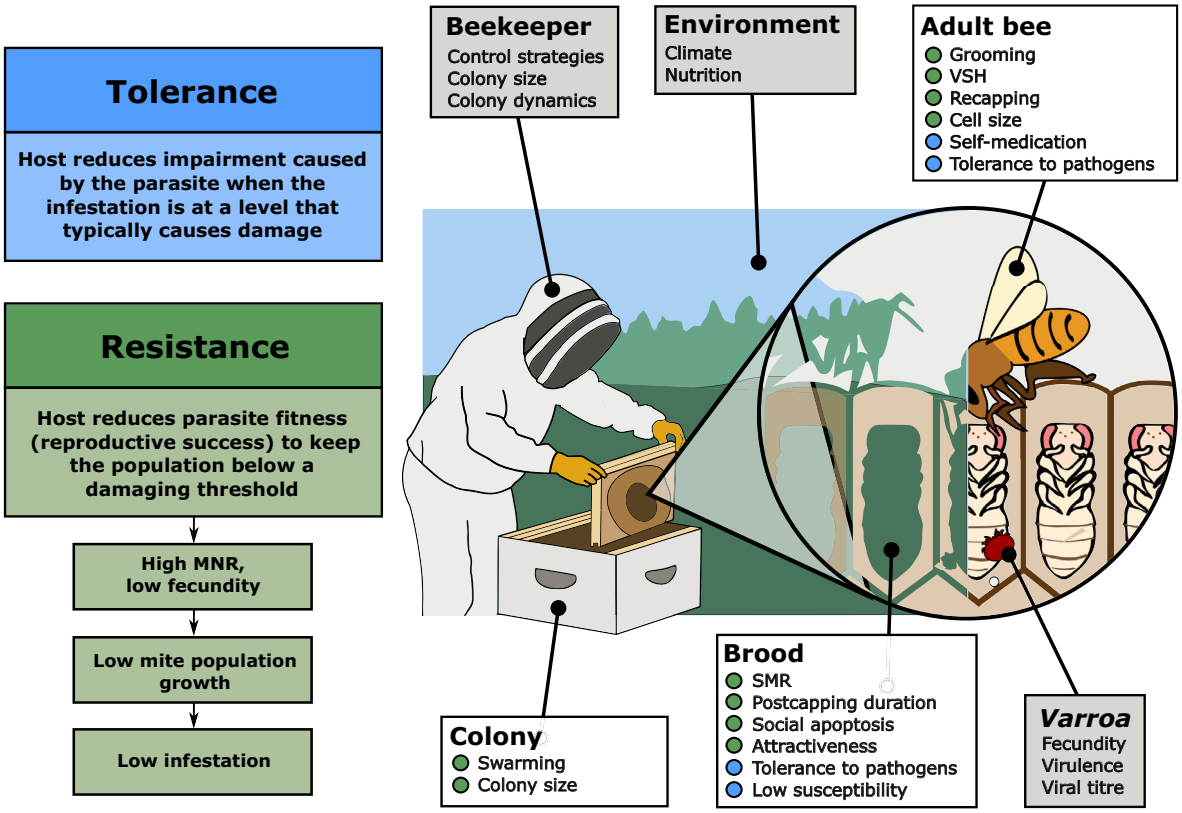

Fig. 4. Overview of host traits and other factors that contribute to the ability of honey bees to survive parasitism by Varroa. Honey bee traits are either resistance (indicated by blue) or tolerance (indicated by green). Non-host factors (grey background) are related to either beekeeping management, *Varroa* adaptations or the environment. Elements in this graphic are adapted from McAfee et al. (2017a, Fig. 1) (Creative Commons Attribution 4.0 International license. https://creativecommons.org/licenses/by/4.0/). SMR, suppressed mite reproduction; VSH, Varroa sensitive hygiene.

three types of molecular markers could be used to select and improve the bee stocks, and the 'omics disciplines have greatly contributed to our understanding of the factors shaping honey bee health (Grozinger and Robinson, 2015; Trapp et al., 2017; Doublet et al., 2017). A multitude of studies investigating similar traits have now been conducted on honey bee populations surviving varroa infestation, which provides opportunities for new perspectives on specific molecular mechanisms and marker-assisted selection (MAS).

3.2. Identifying social immunity pathways

In order to understand whether resistance traits identified in populations of *A. mellifera* share the same genetic basis, we compared all relevant 'omics studies available on this topic. In all, 27 studies were included in the analysis (references in Supplementary Table S1). We also included analyses of HYG, which is not specific to varroa (see Section 2.3), to test whether the genetic basis of this trait and other varroa resistance traits are correlated. We first drew a map of the 16 *A. mellifera* chromosomes based on the size in Mbp from the Amel 4.5 assembly (Weinstock et al., 2006), which enabled us to include older studies. We then extracted locus information of the putative QTLs or SNPs involved in the resistance traits. Whenever locations were given in cM, they were converted to bp based on recombination frequencies reported in Beye et al. (2006) (19 cM/Mb). Given the low genome-wide variation in recombination rate, this ratio was kept constant for all chromosomes. If available and extractable, information from transcriptomic studies was added to the scheme, excluding studies evaluating <25 genes (Supplementary Table S1). We converted Differentially Expressed Genes (DEGs) reported in these studies in old and new Beebase IDs (hymenopteragenome.org, metazoan.ensembl.org) whenever possible and obtained their location on the honey bee genome from the Ensembl Genome database (Kersey et al., 2018) and the Hymenoptera Genome Database (Elsik et al., 2018). This analysis resulted in 159 DEGs found in at least two of the eight studies, of which we could retrieve information on the location on the genome for 118 DEGs.

The most striking result from this literature survey is the lack of overlap in the findings across studies (Fig. 5). This has several potential explanations: (i) the different traits do not involve the same genetic pathways, (ii) the subspecies or populations of *A. mellifera* are different across studies and express the same trait through different molecular pathways, (iii) the protocols used to phenotype the bees are different and (iv) the technologies used are different. False positives (controlled between 1% and 10% depending on the study) may explain some of these differences, but not to the magnitude we observe here.

We expected that different varroa resistance traits may, in part, rely on the same sensory pathways (e.g. olfaction is likely involved in both VSH and HYG, and odorant binding protein 3 expression has been linked to grooming) (Guarna et al., 2017). Indeed, some loci appear to be linked to more than one social immunity trait (Fig. 5). For instance, some of the SNPs from the HYG investigations of Harpur et al. (2019) fall within the locus found by Tsuruda et al. (2012) when investigating VSH. However, more often, studies did not report the same regions to be involved, whether they were investigating the same trait or not.

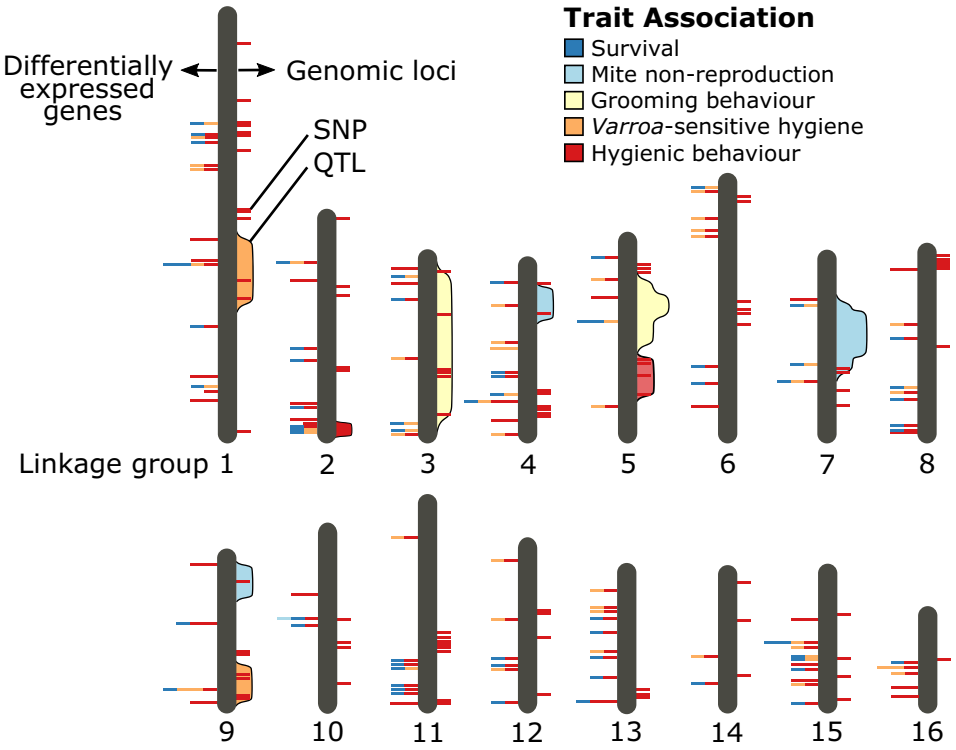

Fig. 5. Comparison of honey bee genomic regions identified by the different 'omics studies. Schematic representation of the genome of *Apis mellifera* showing the approximate location of the markers identified by the genomic studies and differentially expressed genes from transcriptomics studies. Only genes that were differentially expressed in two or more studies are shown, with each bar representing one study. Wider quantitative trait loci bars indicate that the quantitative trait loci was identified in more than one study. Genomic studies included Oxley et al. (2010), Behrens et al. (2011), Arechavaleta-Velasco et al. (2012), Tsuruda et al. (2012), Spötter et al. (2012, 2016), Bourgeois et al. (2015), Lattorff et al. (2015), Conlon et al. (2018, 2019) and Harpur et al. (2019). The details of the genes, traits, and references are presented in Supplementary Tables S2–S4. SNP, single nucleotide polymorphism.

The diversity of *A. mellifera* populations with resistance or tolerance to *V. destructor*, together with the fact that these populations are located in very different geographic regions, suggest that mechanisms may have evolved independently in separate populations. Indeed, reports of convergent evolution for disease resistance in nature are rare (Meyers et al., 2005). Furthermore, duplicated genes, genes involved in the same physiological process, or different genes that achieve the same phenotypic outcome, may be blurring the picture (see below). Despite this potential obstacle, studies on the same trait (e.g. grooming) in distinct *A. mellifera* populations sometimes showed comparable results (Arechavaleta-Velasco et al., 2012; Bourgeois et al., 2015).

Despite tremendous efforts to homogenise protocols (Dietemann et al., 2013; Human et al., 2013), diverse methods and phenotyping protocols are still employed to study a given honey bee trait. Moreover, results of transcriptomic studies vary according to the choice of samples used, including the number of individuals, caste, age and organs or tissue of the bees. Altogether, given these technical differences, it is perhaps not surprising to observe so little overlap between studies.

Ideally, high-throughput analyses performed on the same biological system should yield similar outcomes. However, Fig. 5 clearly shows that this is not the case. Other reports also document that across 'omics fields, variation in results is considerable and transcript and protein abundance often do not correlate (Gygi et al., 1999; Liu et al., 2016). For example, studies on the same trait, population, and with the same sampling protocols, have given very different results, possibly due to differences in genotyping technologies, from QTL mapping using hundreds of microsatellites to high-throughput sequencing using thousands of SNPs (Behrens et al., 2011; Conlon et al., 2018). Altogether, this illustrates the clear need for homogenization and replication in order to tackle biological questions in ways that are less condition-dependent.

3.3. Molecular mechanisms involved in Varroa resistance by honey bees

Although there is a poor overlap of specific molecular markers between studies, the functions associated with such genetic markers and enriched in the different studies show some consistency between the different populations phenotyped. Notably, functions related to neural sensitivity, signal transmission, sensory perception, olfaction, transporter activity, metabolic process and oxidative phosphorylation have been found to be related to VSH and HYG in several studies (Supplementary Tables S2–S4). This suggests that neuronal functions and olfactory pathways play a key role in shaping the behavioural resistance to varroa, very likely via enhanced abilities to detect infested brood. This consistency, despite strong differences in methodologies (molecular techniques, targeted tissues, bee populations, etc.) is quite remarkable and demonstrates the strong link between those biological functions and varroa resistance related to VSH and HYG.

We performed an enrichment analysis on the 159 DEGs identified in at least two different transcriptomic studies of varroa resistance (using DAVID, Huang et al., 2009), but no molecular function and biological process was found to be significantly enriched, partly due to the relatively low number of DEGs (118 annotated) but also the different tissues that were analysed. However, investigating these 159 DEGs, five encode ion channel proteins (GB49268 – glutamate receptor ionotropic kainate 2, GB50159 – neuronal acetylcholine receptor subunit alpha-10, GB42728 – sodium channel protein paralytic, GB51897 – cacophony, GB50377 – two pore

potassium channel protein sup-9) and six function as oxidoreductase (GB49380 – fatty acyl-CoA reductase 1, GB53651 – nitric oxide synthase, GB49878 – probable cytochrome P450 6a14, GB51356 – cytochrome P450 4G11, GB41212 – laccase-5, GB50627 – putative fatty acyl-CoA reductase). Ion channel proteins are interesting since they are located on the membrane of excitable cells (neuron, muscle) and therefore likely involved in behavioural modulation. The prevalence of oxidoreductases suggests a different energy regulation in analysed tissues (including neurons and muscles). The roles of metabolic processes and oxidative phosphorylation in regulating HYG are not known, but are possibly linked to the differential modulation of neuron and muscle activity (Hall, 2012).

3.4. Challenges implementing MAS

The extremely high rates of genetic recombination in the honey bee is both a hindrance and a benefit for identifying genetic markers (Beye et al., 2006; Wallberg et al., 2015). The frequent recombination means that non-causal SNPs will quickly become dissociated from the phenotype. But in rare cases, it could lead to selection for an alternative causative allele. For example, Tsuruda et al. (2012) used a mapping population of VSH x unselected Italian bees to identify one major QTL that was associated with VSH behaviour. When VSH activity and the causative alleles at a key SNP were examined later in Russian stock, the allele that was associated with high VSH activity in the original mapping population was instead associated with lower activity in the Russian population (Kirrane et al., 2015). As the authors indicate, this suggests that a stable recombination event occurred after the two populations diverged. If MAS had been employed using this allele, VSH would theoretically be enriched in one stock and suppressed in the other. This example illustrates well that large scale allelic frequency studies combined with mechanistic confirmation or validating selective breeding experiments will be necessary to identify allelic markers that are reliable enough to be used in MAS across stocks. Although challenging, mechanistic confirmation or validating selective breeding experiments in diverse populations will be necessary to identify allelic markers that are reliable enough to be used in MAS across stocks. However, it is possible that such a 'one size fits all' marker does not exist, if different populations of honey bees are undergoing divergent evolution of varroa resistance traits – an idea that is consistent with the generally poor overlap between 'omics studies discussed above.

Once causal genetic markers for varroa resistance are identified, genetic testing has the advantage of being inexpensive and reliable; therefore, it is an ideal commercial diagnostic test. However, proteomic and transcriptomic technology is becoming increasingly robust and cost-effective, while not suffering from recombination-based signal decay over time. A collaboration of Canadian researchers has developed a biomarker panel of 13 proteins (nine associated with HYG, two with VSH, and two with grooming), and have demonstrated that using these proteins for MAS yields resistance to varroa that is comparable to the leading field selection methods (Guarna et al., 2017). While this study validated biomarker efficacy in an independent population of *A. mellifera*, selection based on expression markers may still be sensitive to extraneous environmental variables. Grooming aptitude, for example, is known to vary with temperature (Currie and Tahmasbi, 2008), and so might its underlying transcript and protein markers.

3.5. Odorant binding proteins (OBPs): a case study to illustrate the poor agreement of expression patterns across HYG studies

Several studies have investigated differential expression patterns in honey bees from hygienic and non-hygienic colonies. Olfaction is widely agreed to be a key biological process enabling HYG (Gramacho and Spivak, 2003; Chakroborty et al., 2015): hygienic honey bees are better at odorant discrimination tasks and detect disease odorants at lower thresholds than non-hygienic honey bees. OBPs are thought to aid in peripheral odorant detection by binding and transporting odorant molecules from the antennal pore to the olfactory receptor neurons, but their expression in non-olfactory tissues suggests they have diverse biological roles. Many differential expression studies involving hygienic and non-hygienic honey bees have identified OBPs within the lists of significant genes and proteins. However, there is a huge diversity in the specific OBPs that have been identified (Table 4). For example, Gempe et al. (2016) found that OBP1 and OBP21 are downregulated in hygienic worker brains, but Boutin et al. (2015) and Scannapieco et al. (2017) found that OBP4 is downregulated in brains and heads. If there is one conserved molecular mechanism for HYG, the researchers theoretically should have identified the same OBPs even across geographically isolated populations. Similar inconsistencies are observed for gene expression in antennae of high and low VSH bees (Mondet et al., 2015; Hu et al., 2017). Some studies examined different tissues across which the same gene is differentially regulated. Confounding factors such as

Table 4
Significantly differentially expressed odorant binding proteins across studies: an illustrative example.

Gene	Accession number (s)	Trait	Detection method	Tissue	Direction of regulation	Reference
obp1	GB55593, GB11135	HYG	Microarray	Brain	Down	Gempe et al. (2016)
obp1	GB55593, GB11135	HYG	QTL	–	–	Oxley et al. (2010)
obp3	GB53371, GB30242	VSH	Microarray	Brain	Down	Le Conte et al. (2011)
obp3	GB53371, GB30242	VSH	RNA-seq	Antennae	Up	Mondet et al. (2015)
obp3	GB53371, GB30242	HYG	RT-qPCR	Head	Down	Scannapieco et al. (2017)
obp3	GB53371, GB30242	GRM	Proteomics	Antennae	Up	Guarna et al. (2017)
obp4	GB53372, GB13587	HYG	RNA-seq	Brain	Down	Boutin et al. (2015)
obp4	GB53372, GB13587	HYG	RT-qPCR	Head	Down	Scannapieco et al. (2017)
obp14	GB46223	VSH	RNA-seq	Antennae	Up	Mondet et al. (2015)
obp14	GB46223	VSH	Proteomics	Brain	Up	Hu et al. (2016)
obp15	GB46224	VSH	Proteomics	Antennae	Down	Hu et al. (2016)
obp16	GB46225, GB16826	HYG	Proteomics	Antennae	Up	Guarna et al. (2017)
obp17	GB46226, GB11092	VSH	Proteomics	Antennae	Up	Hu et al. (2016)
obp18	GB46227	HYG, VSH	Proteomics	Antennae	Up	Hu et al. (2016); Guarna et al. (2017)
obp18	GB46227	VSH	Proteomics	Brain	Up	Hu et al. (2016)
obp18	GB46227	VSH	Proteomics	Hemolymph	Down	Hu et al. (2016)
obp21	GB46230, GB15460	HYG	Microarray	Brain	Up	Gempe et al. (2016)

HYG, hygienic behaviour; VSH, *Varroa*-sensitive hygiene; GRM, grooming; RT-qPCR, real-time quantitative PCR. Accession numbers are from old and new Beebase IDs (hymenopteragenome.org, metazoan.ensembl.org).

phenotyping techniques and instrumentation make it difficult to draw conclusions about why these OBP inconsistencies exist.

Other experimental evidence suggests that there could be multiple olfactory mechanisms underlying HYG. For example, phenethyl acetate acted as a strong HYG inducer in one population (Swanson et al., 2009), but not in another (McAfee et al., 2017b). It could be that honey bees with distinct genetic origins have low response thresholds to different disease odorants, detecting any one of which could be sufficient to elicit the behaviour. This is consistent with the idea that different odorant reception mechanisms, which enable detection of different disease odorant molecules, could underlie the same behaviour.

4. Research gaps and perspectives

The results of the two literature surveys we conducted highlight a great diversity of issues and knowledge gaps regarding the study of varroa resistance in *A. mellifera*. These gaps can be grouped in two main categories: biological and methodological aspects.

4.1. Biological aspects

An elemental step in breeding for specific resistance or tolerance traits is to accurately characterize trait heritability. (Moritz, 1985; Harbo and Harris, 1999b; Danka et al., 2016). Some traits are highly heritable and may indeed provide sustainable solutions to control the mite. This may be the case for MNR, for which empirical evidence has demonstrated high inheritance via drones or queens (Moritz, 1985; Harbo and Harris, 1999a; Danka et al., 2016; Locke, 2016). However, MNR expression within surviving colonies seems complex, as workers from the same subfamilies do not exhibit the trait homogeneously (Beaurepaire et al., 2019a). Generally, the exact inheritance mechanisms behind the different resistance and tolerance traits, as well as the extent of the variation of these mechanisms across individuals, subfamilies, colonies, and populations, are unknown.

Disease resistance and tolerance traits can act at several levels of social organization in honey bees (Kurze et al., 2016), and colony level expression is often overlooked. Honey bees live in complex, crowded societies, where nests are typically headed by one queen and contain a small fraction of reproductive drones, but the whole colony (the 'super-organism') is considered to be the unit of selection (Wheeler, 1928; Seeley, 1989). Due to this simplified view, important traits exhibited by individual workers are often overlooked (Moritz and Crewe, 2018). In fact, given honey bees' mating system, where queens typically mate with over a dozen unrelated drones (Tarpy et al., 2004), colonies are composed of a diversity of worker subfamilies with distinct genotypes that may differ in their responses to stressors (Page and Robinson, 1991). Moreover, within the same subfamily, individuals with identical genotypes may respond differently to parasites and pathogens, according to conditions such as age or environmental factors (Roberts and Hughes, 2014; Dalmon et al., 2019). There is a general consensus that intra-colonial diversity is highly adaptive for the expression of parasite resistance and tolerance, as it may buffer the colony level response threshold (Tarpy, 2003; Schmid-Hempel, 2011). However, more field studies are needed to confirm the effects of this high colony level genetic diversity and the relative expression of varroa resistance traits in the different subfamilies of honey bee colonies.

In addition to the intra-colonial level, the variation of traits between colonies in a given population is of great importance. Under natural conditions, the high admixture of drones in drone congregation areas provides opportunity for mixing of bee genotypes (Baudry et al., 1998; Harpur et al., 2019), and may facilitate the spread of resistance traits. In *A. mellifera*, drone brood is highly attractive to varroa mites (Le Conte et al., 1989). This attribute may enhance the selection of resistance in nature, since only the fittest males are capable of reaching a queen to mate in flight. Interestingly, studies investigating *A. mellifera* resistance traits in surviving populations often report multiple traits of significant value, and high variation across colonies (Locke, 2016). This suggests that it is the accumulation of multiple resistance traits that enables colonies to survive, and not one major trait.

4.2. Methodological aspects

Today, a great range of techniques and protocols have been developed to study resistance and tolerance in *A. mellifera*. Despite the general agreement of the research community to use specific techniques for investigating simple traits (e.g. opening cells to look for recapping), there is currently a lack of clarity on the precision and accuracy of the methods. In addition, the diversity of available techniques for a given trait generally grows with trait complexity. For instance, quantifying even something seemingly simple such as MPG in *A. mellifera* colonies can be very challenging. Several methods to infer the number of parasites infesting honey bee colonies currently exist: counting fallen mites on the bottom board, estimating phoretic mites using samples of adult bees, or counting mites in the brood (Dietemann et al., 2013; Gregorc and Sampson, 2019). Often, only one of these estimates is used to infer the total population of mites infesting a colony, as combining them is too time-consuming. However, due to fluctuations of brood and adult dynamics in space (e.g. across honey bee populations) and in time (e.g. between seasons), the ratio of mites and bees is constantly changing, and no single estimate can correctly predict entire parasite populations. Consequently, these estimates of mites in brood or on adult bees will not correctly assess the complete populations of parasites, unless they are combined with and/or used in parallel with models allowing for inference of spatio-temporal patterns of mite fluctuations (e.g. Calis et al., 1999). A better understanding of the mechanisms underlying each trait and the link between traits may help in finding proxies that could be used to facilitate phenotyping.

Even though the ability of honey bee colonies to survive varroa without mite control stands as the ultimate goal of varroa resistance and tolerance selection, using "survival" as the only phenotype may be dangerous since it may be dependent on many other factors besides varroa. Our literature review revealed that low MPG is a common downstream result in surviving populations. This highlights three important aspects for methodological development: (i) low MPG can result from many different traits operating alone or in combination; (ii) it can also be influenced by many environmental and beekeeping factors; and (iii) standing as a central feature of varroa-resistant populations, evaluating MPG may be the most robust phenotyping method to assess mite resistance. Future research should thus assess whether MPG can be used as a proxy to phenotype varroa resistance when used alone (such as in the Russian bees breeding program; Rinderer et al., 2001, 2005), or whether it needs to be used in combination with the phenotyping of one or several regulatory traits.

With a growing number of *A. mellifera* surviving populations reported (e.g. Kohl and Rutschmann, 2018; McMullan, 2018), there is a great need to simplify and standardize methods to confirming survivorship and characterize underlying mechanisms. To ensure the suitability of these methods, they should be rigorously tested across several honey bee populations and different environments. This could result in the development of a common breeding effort to solve the varroa problem across the globe, or determine that more local approaches have to be conducted. To date, the available data suggests that the latter is most likely, since adaptation to local environments seems to play a determining role in colony survival (Meixner et al., 2015).

4.3. Parasite adaptations might play a large role in host resistance or tolerance

To date, studies on the resistance and tolerance to varroa have largely ignored the role of the parasite itself (Eliash and Mikheyev, 2020). This omission may be due to the fact that the introduced mite populations were considered pseudo-clonal due to the bottlenecks following host shifts and invasion of the parasite globally (Solignac et al., 2005), and therefore not likely to exhibit variation in complex traits. However, more recent evidence shows that *Varroa* spp. have shown that the mite populations are more genetically variable than previously thought (Robertson et al., 2014; Beaurepaire et al., 2015; Dynes et al., 2017; Dietemann et al., 2019), and that they can evolve swiftly in response to selective pressures such as acaricide treatments (Beaurepaire et al., 2017) in an arms race with their host (Beaurepaire et al., 2019b). Although clear links between these genetic findings and phenotypes in the field are currently missing, investigating the mite side of the story will help disentangle the role of the parasite traits in host resistance (Fries and Camazine, 2001; Seeley, 2017).

The relationship between *V. destructor* and *A. mellifera* still remains greatly unbalanced due to continuous human interference (Neumann and Blaquiere, 2016). This disequilibrium affects not only the honey bee, but also the mite populations for which collapsing colonies may represent a dead-end. Thus, an arms race between the host and the parasite should lead to an evolutionary equilibrium, where both species can perpetuate in more balanced ways (Thompson, 1994).

5. Conclusions

The resistance and tolerance mechanisms of honey bees that survive with varroa, whether acquired through natural selection or through selective breeding efforts, span a tremendous range of honey bee behaviour, individual immunity, population dynamics, and relationships with associated pathogens. Moreover, it is very likely that these mechanisms do not operate alone but may function in combination. The importance of specific adaptations may vary across environments. Future research should aim at understanding the potential links between traits, and why so little overlap is found in studies looking at molecular pathways underlying varroa resistance and tolerance. Such efforts will also help develop practical tools to assist selection programs to enhance varroa resistance and tolerance, either by offering molecular markers or by finding proxies of complex traits that could help with phenotyping colonies in the field. Phenotyping efforts and molecular maker development are complimentary approaches, and efficient development of MAS relies on the development of effective and reliable phenotyping tools.

The results of these analyses highlight the need to unify efforts in the research community, while presenting the most promising traits for future efforts in selective breeding for varroa-surviving bees.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2020.03.005.

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