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Three decades of selecting honey bees that survive infestations by the parasitic mite *Varroa destructor*: outcomes, limitations and strategy

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Running title: Selecting honey bees against *Varroa destructor*

Abstract

Despite the implementation of control strategies, the invasive parasitic mite *Varroa destructor* remains one of the principal causes of honey bee (*Apis mellifera*) colony losses in numerous countries. For this reason, the parasite represents a serious threat to beekeeping and to agro-ecosystems that benefit from the pollination services provided by honey bees. Numerous selection programmes have been initiated over the last three decades with the aim of promoting the establishment of balance in the host–parasite relationship and, thus, helping European honey bees to survive in the presence of the parasite without the need for acaricide treatments. Such programmes have focused on either selective breeding for putative resistance traits or natural selection. To date, no clear overview of these attempts has been available, which has prevented building on past successes or failures and, therefore, hindered the development of a sustainable strategy for solving the *V. destructor* problem. In the present study, we review past and current selection strategies, report on their outcomes and discuss their limitations. Based on this state-of-the-art knowledge, we propose a strategy for increasing response to selection and colony survival against *V. destructor* infestations. Developing in-depth knowledge regarding the selected traits, optimising selection programmes and communicating their outcomes are all crucial to our efforts to establish a balanced relationship between the invasive parasite and its new host.

Keywords: *Apis mellifera,* host–parasite relationship, natural selection, resistance, selective breeding, *Varroa destructor*.

Introduction

The western honey bee, *Apis mellifera*, is one of the most valuable pollinators worldwide (Aizen and Harder, 2009; Gallai *et al*, 2009; Hung *et al*, 2018). Over the last few decades, increased honey bee colony losses have been reported, mostly in the Northern Hemisphere (Ellis *et al*, 2010; Neumann and Carreck, 2010; Potts *et al*, 2010b), possibly as a result of a growing number of interacting threats, such as habitat losses, nutritional deficiencies, pesticides, pests and pathogens (Goulson *et al*, 2015; Potts *et al*, 2010a; Vanbergen, 2013).

Among the parasites, the invasive mite *Varroa destructor* is often identified as the main macrobiotic cause of colony losses of *A. mellifera* of European origin in many regions (e.g. Dahle, 2010; Guzman-Novoa *et al*, 2010; Le Conte *et al*, 2010; Morawetz *et al*, 2019; Neumann and Carreck, 2010; van Dooremalen *et al*, 2018). This parasite originates from Southeast Asia, and it shifted from its original host, *A. cerana*, to *A. mellifera* at the beginning of the 20th century, when the latter was imported to the Russian Far East (Crane, 1978; Oldroyd, 1999). The parasite rapidly spread around the world due to the globalised trade in *A. mellifera* queens and swarms (Mutinelli, 2011; Owen, 2017). Only a few areas, including Australia, some regions of Northern Europe and some islands, are still considered to be free of *V. destructor* mites and, thus, safe from the parasite's detrimental impact.

V. destructor is not lethal to *A. cerana* due to the host–parasite co-evolution (Boot *et al*, 1997; Peng *et al*, 1987; Rath, 1999). The reproduction of the parasite is limited to the transient male (drone) brood of *A. cerana*, which restricts the population growth of the mite. In contrast, in *A. mellifera*, the new host, the ability of the parasite to use both the drone brood and the more persistent worker (non-reproductive female) brood leads to high infestation levels (Branco *et al*, 1999; Kraus and Page, 1995; Liebig, 2001). Thus, a large proportion of the colony is weakened by the feeding (Amdam *et al*, 2004; de Jong *et al*, 1982; Ramsey *et al*, 2019; Zaobidna *et al*, 2017) and pathogen vectoring activity (Barroso-Arévalo *et al*, 2019a; Carreck *et al*, 2010; Dainat *et al*, 2012b; Francis *et al*, 2013; McMenamin and Genersch, 2015; Mondet *et al*, 2014; Yang and Cox-Foster, 2007) of the mother mite and its offspring. Upon emergence, the infested individuals do not perform optimally or else they die early, which threatens colony survival and reproduction (Arguello-Najera and Vandame, 2003; Fries *et al*, 2003; Korpela *et al*, 1992; Ritter *et al*, 1984).

Currently, to prevent colony losses due to *V. destructor* infestations, beekeepers rearing European honey bees limit the parasitic pressure on their stocks by implementing control strategies. Such strategies often rely on chemical treatments involving synthetic miticides, organic acids or essential oils (Rosenkranz *et al*, 2010). They may also include biotechnical measures, such as the removal of the preferentially parasitised drone brood. Beekeepers can coordinate these actions within the framework of an Integrated Pest Management strategy (Calderone, 2005; Delaplane *et al*, 2005; Imdorf *et al*, 2003). Strategies based on synthetic miticides are problematic because their residues contaminate hive products (Bogdanov, 2006), and they are also likely to favour the emergence of resistant lineages of *V. destructor* (Milani, 1999; Spreafico *et al*, 2001; Trouiller, 1998). Although treatments involving organic acids have proved effective and do not leave residues when used correctly, they can have negative side effects on honey bee health (Tihelka, 2018). Due to such problems, a growing number of beekeepers are trying to reduce their reliance on chemical treatments (Andrews, 2019; Thoms *et al*, 2018; Underwood *et al*, 2019), which has highlighted the need for alternative and sustainable approaches to control this parasite, including selecting honey bee lineages that survive parasite infestations (Dietemann *et al*, 2012). Favouring the expression of traits that promote the survival of the new host could lead to a balanced host–parasite interaction and so remove the need for human intervention to reduce the parasite population.

The idea of selecting less susceptible colonies emerged shortly after the global invasion of the mite (Büchler and Drescher, 1989; Kulincevic and Rinderer, 1986; Moritz, 1985; Peng *et al*, 1987), following the observation that several populations can survive in the presence of the parasite, without the need for treatments. This is the case for the sub-Saharan African subspecies of *A. mellifera* (Dietemann *et al*, 2009; Fazier *et al*, 2010) and the derived Africanised honey bees found in South and Central America (Moretto *et al*, 1995; Rosenkranz, 1999; Schneider *et al*, 2004). The discovery that some European *A. mellifera* populations used for beekeeping could also better survive *V. destructor* infestation (Büchler, 1994a; Büchler and Drescher, 1989; Morse *et al*, 1991) opened up avenues for human-mediated selection for colony survival and survival-associated traits in the susceptible population most frequently used for beekeeping in the Northern Hemisphere.

Over the last three decades, during which *V. destructor* has spread almost globally, beekeepers and researchers have initiated numerous selection programmes intended to promote the survival of susceptible populations without the need for treatments. The origin and the aim of some selection programmes have previously been described (Büchler *et al*, 2010; Locke, 2016; Rinderer *et al*, 2010). However, an exhaustive overview of the resistance selection programmes implemented to date, as well as of their outcomes, that is, whether they resulted in populations surviving without treatments, has not yet been provided. A discussion of the limitations of the applied strategies is also still lacking. Such gaps in the literature prevent the optimisation of selection programmes and the identification of a sustainable solution to the *V. destructor* problem based on this approach.

In the present study, we provide a comprehensive review of 30 years of scientific and specialised literature, with the aim of filling the identified knowledge gaps in research and selection programmes concerning resistance against *V. destructor*. By compiling and analysing this information, we aim to provide tools for beekeepers, breeders and researchers with varying levels of knowledge of genetics and selection principles, who are either interested in *V. destructor*-resilient honey bees or in the biological aspects of host–parasite relationships. In the first section, we provide an overview of the selection programmes: we discuss the principles and the advancement of the different breeding strategies, their outcomes and whether the selected lineages are available on the market. This summary can be used as a bibliographical resource by scientists and beekeepers who wish to become acquainted with the different approaches available, as well as their pros and cons. To help breeders define their strategy, for each selected trait, we present their assessment methods, their estimated heritability and their effect on colony survival. In the second section of the review, we present the theoretical and practical limitations of the strategies described in the first section, with the aim of identifying gaps in the design of the selection programmes. Based on these considerations, in the third section, we offer recommendations to best design selection programmes in order to more efficiently drive the progress of selection towards colony survival against *V. destructor* infestations. Progress could be achieved not only by developing new selection strategies, but also by applying known albeit often overlooked genetic concepts. Overcoming the challenge posed by the deadly *V. destructor* parasite by means of selection programmes requires the inclusion of a high number of colonies and populations. It also requires programme monitoring, as well as data analysis and publication. This exceeds the capacities of individuals or small groups, and it is only possible if researchers and breeders join forces.

I. Overview and outcomes of selection strategies

The survival of colonies infested by *V. destructor* in the absence of acaricide treatment is a selection goal shared by programmes performing **selective breeding** for traits linked to colony survival (section 1.1) and programmes harnessing **natural selection** (section 1.2). Section 1.1 presents the selected traits, their **heritability** values (h^2) and the results of programmes aiming to selectively breed these traits. Information concerning the evaluation methods used in relation to each trait and bibliographical references supporting or refuting the effectiveness of their selection are provided in the supplementary material (Tables SA–SG). Section 1.2 lists the strategies used to detect and multiply survivor colonies in programmes based on natural selection. Section 1.3 summarises the outcomes of the selection strategies some three decades after initial attempts to achieve balance in the host–parasite relationship.

1.1 Selective breeding of heritable traits linked to colony survival

Increased survival can be the result of host tolerance (Figure 1), the mechanisms of which limit the severity of the disease (varroosis) induced by a given mite burden. As a result, colonies are relatively unaffected by *V. destructor* infestations. Survival can also stem from host resistance (Figure 1), which confers defence mechanisms on the colonies, thereby reducing the parasitic pressure (Harbo and Harris, 1999b; Råberg *et al*, 2009), or by a combination of the two mechanisms. To allow colonies to survive without treatment against *V. destructor*, programmes inspired by those mechanisms likely responsible for the low infestation rates of the original host, *A. cerana*, or of naturally surviving *A. mellifera* populations have been initiated to select for colony resistance traits (Büchler *et al*, 2010; Rinderer *et al*, 2010; Uzunov *et al*, 2017). In contrast, selection programmes targeting tolerance traits have not been reported to date. The principle behind selecting for resistance lies in the assumption that colonies with low infestations survive better (Carreck *et al*, 2010; Döke *et al*, 2015). The main challenge faced by such selection programmes is, therefore, identifying traits that limit the mite population that have a sufficiently high heritability to be effective regardless of the environment. It is only in this way that the selected colonies can survive irrespective of the management type, season or location. Resistance traits affect different stages of the life cycle of *V. destructor*. They are either linked to its reproduction within the honey bee brood (section 1.1.1) or to the survival of the mite on the adult host (section 1.1.2), which is used by the parasite for feeding and transportation (Rosenkranz *et al*, 2010).

1.1.1 Traits that reduce *V. destructor* infestation within brood

1.1.1.1 Suppressed mite reproduction (SMR)

Early studies concerning the biology of *V. destructor* identified reduced reproductive output of foundress mites in naturally surviving populations, for example, in Africanised honey bees, in which it has been associated with low mite infestation, likely explaining colony survival (Camazine, 1986; Mondragon *et al*, 2005; Rosenkranz, 1990). This trait, which was later termed suppressed mite reproduction (SMR), was included in several selection programmes with the aim of limiting the infestation level in susceptible host colonies (Table SA). To achieve this, the fertility of mother mites is assessed among the worker brood of the tested colonies.

SMR was found to have low to high heritabilities ranging between $h^2 = 0.06$ and $h^2 = 0.63$ depending on the investigated population (Table A), which indicates that this trait is partly influenced by the genome of the host, although large heritability differences between populations may limit the likelihood of achieving ubiquitous genetic progress. In addition, the link between SMR and infestation levels is not universal (Table SA), indicating that selection for SMR may only lead to successful resistance selection in particular populations. To the best of our knowledge, no **response to selection** for this trait nor survival data regarding untreated SMR lineages have been published to date, which makes it difficult to evaluate the success of SMR selection programmes.

1.1.1.2 Varroa sensitive hygiene (VSH)

In *A. cerana*, adult workers remove most of the immature mite-infested worker brood (Peng *et al*, 1987; Rath and Drescher, 1990). This defense behavior has also been identified in naturally surviving *A. mellifera* populations (Allsopp, 2006; Locke, 2016), and it has been termed *Varroa* sensitive hygiene (VSH). VSH has been identified in susceptible *A. mellifera* populations (Boecking and Drescher, 1991) and, thus, appears amenable to selection. Several methods have been developed to measure the ability of adult workers to remove infested brood, and they have been implemented in various programmes (Table SB).

The repeatability of VSH measurements has been shown to be fairly high, with correlations between repetitions on the same colonies of about r = 0.6 being identified (Boecking, 1994). The heritability value of VSH was investigated in just a single study and found to be moderate (Table A). This result suggests that, at least in some populations, VSH can be reliably measured and, further, that it is transmissible, with both these characteristics being required for a selectable trait. Some publications mention negative correlations between VSH and infestation levels, although no response to selection for VSH has been published to date (Table SB). Survival data concerning colonies selected for VSH in untreated conditions have not been published either. More information is, therefore, required to evaluate the prospective success of selecting this trait in beekeeping conditions.

1.1.1.3 Uncapping–recapping

The uncapping and recapping mechanism is gaining increasing attention as a resistance trait. This behaviour has been identified, along with VSH, in several naturally surviving populations. In *A. cerana*, in an artificial infestation experiment, only about 10% of infested cells were emptied, while in another 40%, only the mite had disappeared (Rosenkranz *et al*, 1993). Later, in a comparison of Africanised and *A. m. carnica* colonies with regards to VSH, it was determined that three to seven days after the initial artificial infestation, the absence of only the introduced mite was more common than the removal of both the mite and pupae (Aumeier and Rosenkranz, 2001; Aumeier *et al*, 2000). Comparable findings were later obtained in *A. m. scutellata* (Cheruiyot *et al*, 2018), and high recapping rates were identified in naturally surviving *A. m. capensis* and Africanised honey bees (Martin *et al*, 2019b). As the cells were experimentally sealed after infestation, it can be hypothesised that they were opened by adult workers, which induced the exit of the mite, before later being recapped. These observations gave rise to the notion that the opening (uncapping) and re-sealing (recapping) of brood cells by workers could be a resistance trait also capable of interrupting or disturbing *V. destructor* reproductive cycles in European honey bees. Thus, evaluation protocols for this trait were proposed (Table SC). In contrast to VSH, this behaviour does not require the sacrifice of brood, meaning that it is less costly for the colony.

Negative associations between the frequency of uncapping–recapping and *V. destructor* infestation levels in brood have been reported (Table SC). Although this is encouraging, heritability estimates are not currently available to determine whether this trait is a good candidate for selection. To the best of our knowledge, no honey bee selection programmes have included uncapping–recapping rates to date, so it is not yet possible to evaluate the potential of this trait with regards to limiting infestation and colony mortality.

1.1.1.4 Hygienic behaviour against dead brood

Hygienic behaviour is a trait linked to the removal of dead brood that is known to drive resistance to both chalkbrood and American foulbrood (Gilliam *et al*, 1988; Spivak and Reuter, 2001; Woodrow and Holst, 1942). Following the hypothesis that the removal of parasitised pupae could be performed more efficiently in colonies that exhibit a high ability to remove dead brood, it has also been used in selection programmes for resistance against *V. destructor*. Different methods, assessing the time needed to remove experimentally killed brood, have been used to measure this trait (Table SD). Their common advantage, when compared to VSH, resides in the low workload required to obtain **phenotypic values**, as no mite manipulation is needed.

The heritability values vary between $h^2 = 0.02$ and $h^2 = 0.65$, with the majority of studies reporting values between 0.2 and 0.6 (Table A). These relatively high values suggest that hygienic behaviour can be successfully selected in most populations. The genetic correlation between VSH and the removal of freeze-killed brood was found to be $r = 0.61 \pm 0.51$, while the phenotypic correlation was $r = 0.11$ (Boecking *et al*, 2000), which suggests that selection for colonies that are hygienic towards dead brood could represent an indirect means of selecting for VSH. However, the relation between hygienic behaviour against dead brood, the infestation level and the survival rate varies among populations (Table SD). Indeed, selection for hygienic behaviour against dead brood did not increase resistance against *V. destructor* in all populations.

1.1.1.5 Reduced post-capping stage duration

A reduced post-capping stage duration is another potential trait for selection capable of limiting the number of viable female *V. destructor* offspring per reproductive cycle. A short post-capping duration has been identified among naturally surviving *A. cerana* (Rosenkranz and Engels, 1994), *A. m. capensis* in South Africa (Moritz and Hänel, 1984), Africanised honey bees in South America (Rosenkranz, 1990) and, more recently, albeit at a lower level, naturally surviving European honey bee populations (Oddie *et al*, 2018b). This trait has, therefore, been recorded using several methods for selection in non-resistant populations (Table SE).

The heritability estimates for this trait are above 0.20 (Table A), which should enable a positive response to selection. As it has been shown to depend on the origins of both the worker brood and nursing honey bees (Bienefeld, 1996b; Kralj, 1998; Langenbach, 1991), this trait may depend on several mechanisms, which could be selected concomitantly. Although a link between shorter post-capping durations and lower infestation levels has been reported (Table SE), we found no records of successful selection programmes for enhanced colony survival based on this trait.

1.1.2 Trait reducing *V. destructor* infestation on adults: grooming

Aside from the removal of mites from brood cells, the removal of mites adhering to adult workers by means of auto- or allo-grooming occurs in some honey bee populations. During grooming, workers brush mites off their bodies or those of their nest mates using their legs, and they may also bite them with their mandibles, thereby causing injuries to the mites, especially to their legs (Pritchard, 2016). A high propensity for grooming may partly explain the resistance of *A. cerana* (Büchler *et al*, 1992; Peng *et al*, 1987). This behaviour has also been identified in naturally surviving *A. mellifera* populations such as Tunisian *A. m. intermissa* (Boecking and Ritter, 1993; Ritter *et al*, 1990) and Africanised honey bees in Mexico (Arechavaleta-Velasco and Guzman-Novoa, 2001; Mondragon *et al*, 2005). Laboratory experiments have been conducted (Hamiduzzaman *et al*, 2017; Hoffmann, 1996; Invernizzi *et al*, 2016; Kirrane *et al*, 2012; Szabo *et al*, 1996; Thakur *et al*, 1997) and encouraged the implementation of grooming as a field trait (Table SF).

The published heritability estimates concerning grooming behaviour vary significantly between $h^2 = 0$ and $h^2 = 0.71$ (Table A). The suitability of this trait for selection is also controversially discussed, with different relations to infestation levels being noted depending on the population being recorded (Table SF). The grooming intensity and the percentage of injured mites have been considered in several selection programmes (Table SF). Lacking data regarding the outcomes of these programmes may explain why there is a lack of consensus concerning the utility of grooming-related selection traits (Bienefeld, 1996a; Liebig, 1996).

1.1.3 Low infestation levels or growth rates as selected traits

Some naturally surviving honey bee populations exhibit low infestation levels (e.g. Martin and Medina, 2004; Rath, 1999; Strauss *et al*, 2014), and a link between infestation levels and the mortality of susceptible *A. mellifera* colonies has been documented (e.g. Dainat *et al*, 2012b); therefore, the selection of colonies with low infestation levels has been performed by several programmes. As the infestation level is directly related to the parasitic pressure, the implementation of this trait does not require prior knowledge of the underlying mechanisms. Infestation levels or changes in them between two points in time, which reflect parasitic population growth rates, have been measured using several methods in order to identify colonies to be selected (Table SG).

Depending on the host population, estimation method and study, the heritability values for the infestation level or its growth rate range between $h^2 = 0$ and $h^2 = 0.54$ (Table A), with most values within the range being compatible with selection ($h^2 > 0.2$). The results of the few documented cases (Table SG) suggest that selection for infestation growth rates is possible, although these rates may fluctuate depending on the test location, which renders the outcome of selection uncertain. To the best of our knowledge, only a single divergent selection field experiment has reported genetic progress for this trait (Table SG); however, no corresponding data concerning survival in untreated conditions were found.

1.2 Taking advantage of naturally selected surviving populations

Strategies based on natural adaptation to environmental conditions have been proposed to identify colonies capable of surviving infestations by *V. destructor*. This approach follows a ‘black box’ principle: whatever the survival mechanisms are, only the outcome, survival rate and ability to reproduce select the colonies used to generate the following generation. This can result in the joint selection of multiple mechanisms favouring colony survival, including tolerance mechanisms. Selection based on survival also has the advantage of requiring only a limited workload to identify the colonies to be selected: those that survive in the absence of acaricide treatments. Genotype–environment interaction mechanisms, which have been shown to favour the survival of local rather than translocated lineages (Büchler *et al*, 2014), can also be expressed in the population when the selected stock is used locally, in addition to additive genetic effects. Two main strategies for starting natural selection-based programmes are mentioned in the literature. First, surviving populations have been obtained by challenging untreated stock with the parasite, and the surviving colonies have been multiplied as beekeeping stocks or for introgression into local populations (section 1.2.1). Second, colonies from stock with known survival abilities have been imported with the same objectives (section 1.2.2).

1.2.1 Letting natural selection act on local populations

The generation of a surviving population by means of natural selection requires the exposure of an initial stock of *A. mellifera* colonies to *V. destructor* selection pressure through the interruption of varroacide treatments. Beekeeping later carries on with the surviving colonies. Research institutes and beekeepers have created several such populations. For instance, the surviving population maintained in Avignon, France, was constituted between 1994 and 1998 by gathering colonies that had not been treated for more than two years (Le Conte *et al*, 2007). It consists of approximately 20 colonies, and it is studied within the framework of research programmes (Le Conte and Mondet, 2017). A second French surviving population established in the Sarthe region is also reported in the literature (Le Conte *et al*, 2007; Locke, 2016). Another famous example is the so-called 'Bond' population, which was created at the southern extremity of Gotland Island, Sweden. Some 150 colonies of mixed genetic origin infested with a standardised number of mites were left without treatment from 1999 (Fries *et al*, 2006). During the first three years, increasing colony mortality rates were recorded, leading to a population bottleneck (Lattorff *et al*, 2015), although surviving colonies multiplied by natural swarming (Fries *et al*, 2003; Fries *et al*, 2006). The colony mortality rates decreased over the following years (Fries *et al*, 2006). The survival of the Gotland population was likely to be at least partly genetically determined. Indeed, colonies originating in Gotland that were translocated to Germany still showed lower colony sizes and lower infestation levels than the local *A. m. carnica* colonies (Schnell, 2007), despite the different environment. The Gotland population comprised 20 to 30 colonies in 2015 (Locke, 2016). It is still monitored for research purposes, although it is not used commercially. Due to the increasing density of non-resistant colonies in the surrounding environment, the experimental population recently experienced increasing infestation levels and, from 2017 onwards, it was treated as a precautionary measure in order to decrease the risk of losing a stock of such scientific importance (Dietemann and Locke, 2019). Although it is not known whether this population would have perished without these treatments, the unusual increase in infestation rates raises a question concerning the long-term resilience of populations that have been through such a severe bottleneck.

A similar programme was initiated by a beekeeper near Toulouse, Southern France (Bandi, 2019). A population of 268 local colonies was left untreated from 1999 onwards, following the 'live and let die' approach (Bond test) of the experiment performed in Gotland. Following the loss of two-thirds of the colonies during the first few years, the best surviving colonies were multiplied and, as the infestation levels remained low, could be kept without acaricide treatments (Kefuss *et al*, 2016). Although no current survival data are available for this population, a commercial activity involving queen production and sales has been developed (www.apiculteur.wordpress.com). Colonies headed by queens from the untreated population from the Toulouse region were compared to other European stocks (some having been selected for resistance mechanisms) in a survival test conducted on a Croatian island, and they were found to exhibit one of the highest survival rates seen in untreated conditions (Büchler *et al*, 2003). This may indicate the survival of this population to involve a genetic component. Many more examples of surviving populations have been reported (Table SH), indicating that natural selection after treatments have been stopped could, under certain conditions, result in the establishment of balance between the host and the parasite.

Stopping treatments on susceptible colonies and so letting them die may not be economically viable. An alternative approach is to identify the fittest rather than the surviving colonies, that is, selecting the colonies with the highest chance of survival before mortality occurs. Following such a 'black box' approach, two populations of untreated colonies were selected in the Netherlands (Blacquière *et al*, 2019). The selection was based on high winter survival levels, but also on traits such as good spring development and the production of drone brood. A trade-off is in general observed between drone production and colony survival in unselected colonies (Kraus *et al*, 2007); therefore, simultaneously selecting for both could be a way of obtaining vital, surviving colonies. Colonies that do not satisfy these vitality criteria are removed from the programme, and they can be managed according to conventional beekeeping practice. The remaining colonies were divided in the spring, and they produced naturally mated offspring at an isolated location so as to promote the concentration of genes favouring colony survival among the population (Kruitwagen *et al*, 2017; Panziera *et al*, 2017). This approach enables the selection of surviving colonies and favours colonies of sizes compatible with apicultural activities. To date, no results concerning the survival and performance of these stocks in beekeeping conditions have been published, which prevents the evaluation of the effectiveness of this approach. One of these stocks has Gotland queens among its ancestors (Kruitwagen *et al*, 2017), which may explain part of the observed resistance independently of the selection procedure.

1.2.2 Importing naturally selected stock

The outcome of a programme designed to obtain surviving colonies depends on the starting population. In order to maximise the probability of success, some selection programmes chose to import queens from foreign surviving populations. For instance, in the United States of America (USA), a programme was based on honey bees originating from the Primorski area (Russian Far East): this population had been exposed to *V. destructor* since the beginning of the 20th century and, therefore, had potentially had more time than other populations to develop defence mechanisms (Danka *et al*, 1995). The first observations in Russia did indeed show that the colonies had low *V. destructor* infestation; however, the inspected colonies were treated by the beekeepers and expressed developed swarming behaviour, while no feral colonies were identified in the sampling region (Danka *et al*, 1995). The occurrence of feral colonies could have confirmed the survivability of this population in the absence of varroacide treatments. As a result, it was initially unclear whether genetic resistance mechanisms were involved. Queens were nevertheless imported between 1997 and 2000 to the USA (Harris *et al*, 2002; Rinderer *et al*, 1997), this was followed by an evaluation, selection and multiplication programme of colonies with lower infestation levels when compared to a local Italian stock (Rinderer *et al*, 2001b; Rinderer *et al*, 2001c; Rinderer *et al*, 2000; Rinderer *et al*, 1999). The resulting lineage was made available to beekeepers across the USA (www.russianbreeder.org). Successive generations of offspring colonies did exhibit the ability to keep *V. destructor* populations at lower levels than the controls (de Guzman *et al*, 2007; de Guzman *et al*, 2019; Rinderer *et al*, 2001a; Tarpy *et al*, 2007), and they also required fewer varroacide treatments (Ward *et al*, 2008). In Canada, colonies with partial Russian ancestry were left untreated in a survival experiment (Robertson *et al*, 2014). Following high colony losses during the first year of the programme, the surviving colonies were multiplied and queens produced for commercial purpose (www.saskatraz.com). In the northern USA, a commercial population of partial Russian ancestry has been managed without treatments for 20 years (Webster, 2005a; Webster, 2005b; Webster, 2019).

In the South of France, in 1993, a commercial beekeeper imported queens from *A. m. intermissa*, an African population for which survival abilities have been documented in its natural range (Boecking and Ritter, 1993; Ritter *et al*, 1990). The colonies originating from the imported queens exhibited better survival than local susceptible controls when left untreated (Kefuss *et al*, 2004). The offspring from these queens were reared until 2004, hybridising with locally kept honey bees, yet still exhibiting resistance when compared to the control colonies (Kefuss *et al*, 2004). In 1989, European beekeepers investigated the potential of using the genetic material of another population of surviving African honey bee, namely *A. m. monticola* from Kenya, to favour resistance against *V. destructor* in susceptible stock (Osterlund, 1991). Semen and eggs were imported from Kenya to Sweden; queens were reared from the eggs and then inseminated with the semen to obtain pure *A. m. monticola* colonies (Osterlund, 1991). The offspring queens were later crossed with Buckfast and *A. m. ligustica* lineages and then tested in beekeeping conditions (Osterlund, 2001). Some colonies from this lineage were maintained without treatment in isolated apiaries (www.elgon.es); however, due to the lack of comparison to controls, the genetic origin of the observed survival was not proven. Colonies originating from this programme are today reared by several commercial European Buckfast beekeepers for honey or queen production, but, to the best of our knowledge, neither the survival nor the resistance to *V. destructor* of these lineages has been documented.

The importing of queens, either belonging to surviving populations or obtained by selective breeding for resistance traits, appears to be a promising approach in terms of starting new programmes in other regions and saving the time required to detect resistant colonies among the local population at the beginning of the process. However, such an approach is fraught with drawbacks (section 2.3.3), which is why imports are not recommended.

1.3 Outcomes of resistance selection programmes: are we on the right path?

The survival of naturally selected *A. mellifera* in the presence of *V. destructor* is possible, even in originally susceptible honey bee populations (section 1.2). Several traits linked to lower colony infestation levels in such surviving populations (section 1.1) could potentially help to increase host resistance if implemented in selective breeding. However, in Europe, within the native range of *A. mellifera*, directed selection for honey bee resistance has not resulted in the better survival of untreated colonies (Neumann and Blacquière, 2017). In Northern America, selected populations from the hygienic, VSH and 'Russian' lines may have a better resistance potential and so are used by beekeepers in several regions (Danka *et al*, 2013b; Rinderer *et al*, 2010). Survey data have indicated lower colony losses in operations rearing resistant stocks of *A. mellifera* when compared to operations rearing only non-resistant stocks (Haber *et al*, 2019). However, high colony losses attributed to *V. destructor* are still reported in the USA (Kulhanek *et al*, 2017). Therefore, to date, selection has not resulted in large-scale, sustainable host–parasite equilibrium.

The limited impact of selection in relation to solving the *V. destructor* problem is surprising given that the majority of the current tests applied to evaluate resistance traits in directed selection programmes were first described more than two decades ago (Hoffmann, 1996; Ifantidis, 1983; Ifantidis, 1984; Koczy-Lorenz, 1986; Naglitsch, 1985; Rademacher, 1985; Rehm, 1988; Ruttner and Hänel, 1992). The reported heritabilities of resistance traits were often high, and their selection should have led to a genetic improvement in the selected stock. However, very little proof of genetic progress, which could serve as a commercial argument for the spread of resistant lineages, is available. Selection responses have been determined for several production or behavioural traits in various populations, indicating that colonies suitable for beekeeping can be obtained through relatively simple selection procedures (Altaye *et al*, 2019; Bar-Cohen *et al*, 1978; Calderone and Fondrk, 1991; Mackensen and Nye, 1969; Manning, 1996; Moritz *et al*, 1987; Wilde *et al*, 2011). The heritability values of these traits are comparable to those of resistance-associated traits (Koffler *et al*, 2016). Thus, following their selection and provided that they do indeed confer resistance, the host–parasite relationship should have become more balanced, while colony mortality due to the mite should have decreased.

Beekeeping is not the only livestock-rearing activity plagued by the negative impact of parasites. In other livestock species, breeders have also selected for resistance against biotic threats. The factors that allow for genetic progress in these species might also be useful in terms of identifying obstacles to progress in relation to honey bee selection against *V. destructor*. Selection for resistance against helminthic parasitism in small ruminants, for example, presents similarities to selection for resistance against *V. destructor*. Indeed, naturally resistant populations have been identified (Zvinorova *et al*, 2016) and, in parallel, significant genetic progress has also been achieved with initially susceptible stocks (Bisset *et al*, 2001; McManus *et al*, 2014; Stear and Wakelin, 1998) despite both the moderate heritability of infestation levels (Pickering *et al*, 2012; Zvinorova *et al*, 2016) and the polygenic origin of this trait (Crawford *et al*, 2006; Moreno-Romieux *et al*, 2017). Individual resistance can be accurately estimated on the basis of the infestation level, even if animals are kept in groups (Aguerre *et al*, 2018), which indicates that the infestation level is a robust selection criterion in small ruminants. The calculation of **breeding values** was later facilitated by the implementation of genomic tools (Pickering *et al*, 2013), thereby limiting phenotyping costs. This indicates that, under the right circumstances, the human-mediated selection of hosts can be successfully applied so as to limit the damage caused by parasites or herbivores; therefore, the reason this is not the case in the honey bee–*V. destructor* relationship needs to be verified.

In the following section, we analyse the limitations of the currently available selection strategies, which hinder progress towards the breeding of honey bee lineages capable of surviving infestations by *V. destructor*.

II. Limitations of selection strategies

The limited contribution of selection to large-scale increased colony survival may be due to several reasons. In addition to the biological characteristics of *A. mellifera* rendering honey bee selection a complex process (section 2.1), it may also be that the traits selected do not result in increased colony survival (section 2.2), that survival depends on other factors than the expression of resistance traits (section 2.3) or that the strategies are not implemented efficiently enough by beekeepers (section 2.4).

2.1 Constraints of honey bee reproductive biology for selection

When compared to other species, selecting *A. mellifera* is a difficult task due to its reproductive and genetic characteristics. The queen performs nuptial flights, which span a wide distance (Jensen *et al*, 2005; Neumann *et al*, 1999; Peer, 1957; Peer and Farrar, 1956; Ruttner and Ruttner, 1972). This implies that controlling mating is challenging and, therefore, either requires isolated mating stations covering the flight range so as to exclude mating with sexuals from unwanted genetic backgrounds (Gregorc *et al*, 2008) or artificial insemination, which allows for the precise control of **sires** and **dams** (Cobey *et al*, 2013).

The appropriate design of structured honey bee selection programmes, enabling the calculation of heritabilities and reliable breeding values, is crucial in terms of generating and monitoring genetic progresses. However, such strategies are difficult to implement due to the complexity of honey bee genetics. As the mating of a honey bee queen involves many drones (Koeniger *et al*, 1979; Kraus *et al*, 2004; Roberts, 1944), a colony is a heterogeneous assembly of worker subfamilies (groups of super- or full-sisters) rather than a single animal, as is the case for other farming animals. As a result, if phenotypes are recorded at the colony level, breeding values need to be calculated for a single distinct individual, namely the queen of the colony (i.e. the dam of the workers). Added to the multiple matings and the haplodiploid reproductive system (Grimaldi and Engel, 2005) of the honey bee, which also impact the establishment of a relatedness relationship between individuals, these biological specificities lead to more complex breeding value estimations when compared to other animal species (Bienefeld *et al*, 2007; Brascamp and Bijma, 2014; Brascamp and Bijma, 2019). The calculation of reliable breeding values, therefore, requires complex algorithms and knowledge of both animal genetics and honey bee biology. This complexity may explain why the calculation of breeding values has only been performed in a limited number of countries to date.

2.2. Relevance of selected traits

The extent to which the traits employed in resistance selection confer an advantage against *V. destructor* is still poorly understood and mostly hypothetical. To date, the traits or combination of traits that render a colony resistant have not been empirically determined. This gap in our understanding of resistance mechanisms is due to the complexity of the experimental designs required to test the role and importance of the suspected traits in terms of providing resistance and, ultimately, ensuring colony survival. The prerequisites for such tests are honey bee lineages expressing different trait combinations at different intensities so that a comparative study can be performed. This has not been achieved to date due to the difficulty of generating such lineages. The multi-year duration of the tests required due to the high longevity of honey bee colonies and the time required for *V. destructor* to exert an impact on colony survival add to the challenge. Alternatively, the results of ongoing selection programmes can be used to study the effects of specific traits on resistance and survival, although it is often unclear whether the selection of traits does indeed lead to better colony survival.

In the following sections, we compile the information available regarding the traits presented in Part I, which could limit their benefit in relation to colony survival, either because the traits have only a limited impact on colony infestation or survival (section 2.2.1) or because environmental factors have a large influence on the expression of the traits (section 2.2.2).

2.2.1 Missing links between traits and survival

2.2.1.1 Suppressed mite reproduction

SMR is likely to be mainly influenced by host factors. Indeed, a cross-fostering experiment involving queens exchanged between colonies with either high or low mite reproduction rates revealed that the SMR values changed according to the origin of the new queen (Harris and Harbo, 2000). However, SMR also depends on many honey bee- or mite-related factors: it is influenced by the reproductive ability of the mite entering the cell (Bienefeld *et al*, 1998; Fuchs, 1994; Harris and Harbo, 1999; Häußermann *et al*, 2019; Locke *et al*, 2012), by the origin of the brood (Conlon *et al*, 2018; Frey, 2009), by the brood quantities available (Locke and Fries, 2011) and by the behaviour of adult workers, including VSH (see section 1.1.1.2) (de Guzman *et al*, 2008; de Guzman *et al*, 2015; Harris *et al*, 2010; Harris *et al*, 2012; Kirrane *et al*, 2015; Kirrane *et al*, 2011; Kirrane *et al*, 2018) and uncapping–recapping behaviour (Oddie *et al*, 2018a) targeting infested cells. SMR has also been shown to fluctuate according to the parasitic pressure: at higher infestation levels, the number of offspring per mite tends to decrease (Eguaras *et al*, 1994), which may negatively impact the repeatability of trait measurement. SMR is influenced by many complex factors, meaning that its contribution to limiting *V. destructor* infestation levels is difficult to establish.

2.2.1.2 Varroa sensitive hygiene

VSH is a complex trait that depends on several factors. It is triggered in response to odour cues emitted by infested pupae (Mondet *et al*, 2016; Wagoner *et al*, 2018) and, possibly, by *V. destructor* (Martin *et al*, 2002). The removal of infested brood occurs in cases of both reproducing and non-reproducing mites (Harris *et al*, 2010), although it has been found to be more frequent when foundresses reproduced (Harris *et al*, 2012). In addition, if VSH kills immature mites (Boecking, 1994), the fate of the foundress mite that parasitised the removed host is not known. It could be killed (see section 1.1.2) or else it could escape and remain alive to invade another brood cell. The complexity of VSH and its potential link to SMR limit our understanding of the mechanisms involved and their contribution to colony resistance, rendering VSH a trait that is difficult to implement in selection. It is also unclear whether VSH can be directly linked to a limitation of the parasitic load. It has not been observed in all surviving populations, which casts doubt on its role in promoting survival (Locke, 2016; Panziera *et al*, 2017). In Africanised honey bees, the influence of VSH on the observed survival rate is controversial, with some studies indicating that this trait could contribute to the limitation of the infestation rate (Guzman Novoa *et al*, 1999; Vandame, 1996), whereas another concludes that it is not a key resistance factor (Aumeier *et al*, 2000). This suggests that VSH has either no influence on survival or that it requires an association with other traits in order to have an effect. In addition, VSH was mainly observed towards the worker brood, while it was only found at low levels towards the drone brood (Boecking, 1994; Harris, 2008). As *V. destructor* preferentially infests the drone brood (Fuchs, 1990) and is also able to produce more viable offspring due to the longer post-capping duration seen in this brood (Al Ghamdi and Hoopingarner, 1997), the contribution of VSH to limiting the infestation level may be lower in colonies rearing many drones, for instance, drone-producing colonies in selection programmes.

In addition, infestation levels increase throughout the season, which leads to a higher frequency of multiple infestations (section 3.2.2). In such a situation, the VSH expression increases (Boecking and Drescher, 1992). The impacts of fluctuating infestation levels on VSH may explain why the measurement of this trait was found to require up to five repetitions to obtain reliable data (Villa *et al*, 2017). The costs involved are likely to restrain the assessment of this trait in field conditions.

2.2.1.3 Uncapping/recapping

The suitability of uncapping–recapping behaviour as a selection trait remains unknown. The impact of this behaviour on the interruption of *V. destructor* reproduction has been shown at the cell level (Oddie *et al*, 2018a), although its influence is still unclear at the colony-infestation level. It has been observed that uncapping/recapping did not very selectively target infested cells (Harris *et al*, 2010). In addition, the results from two studies (Cheruiyot *et al*, 2018; Rosenkranz *et al*, 1993) suggest that the mites easily leave the cell after the cell cap has been opened by inspecting workers, without brood removal. This would interrupt their reproductive cycle. In contrast, in a previous study, it was observed that, during the removal of infested brood by workers, *V. destructor* mites only tended to leave the cell when the pupae was about to be completely removed (Boecking, 1994). Thus, the mite reproduction might not be interrupted in all cases. The mite's willingness to leave its cell once opened may be influenced by previous disturbances in its life cycle or by temperature and humidity conditions. Uncapping–recapping may, therefore, provide different resistance levels, depending on other associated traits.

2.2.1.4 Hygienic behaviour against dead brood

Recent publications have shown that the level of hygienic behaviour towards dead brood is often not a good predictor of the infestation level (Leclercq *et al*, 2018a; Leclercq *et al*, 2018b). An explanation for the low removal response against infested cells seen in some honey bee lines selected for hygienic behaviour could be that specific chemical stimuli trigger the removal of either dead, diseased or infested brood (Danka *et al*, 2013a; Harbo and Harris, 2009; Mondet *et al*, 2016; Schoning *et al*, 2012; Spivak, 1996; Swanson *et al*, 2009), possibly leading to differences in the recruitment of workers specialised in removal tasks (Gempe *et al*, 2016; Theraulaz *et al*, 1998; Wagoner *et al*, 2018). Colonies selected for hygienic behaviour may, therefore, not necessarily efficiently remove infested cells. Indeed, correlation between the removal of freeze-killed brood and the removal of infested brood cells was found when the brood was infested by two mites (Boecking, 1994), but not when the brood was infested by a single foundress mite (Boecking and Drescher, 1992). Thus, hygienic behaviour might only act as a defence mechanism against *V. destructor* in the case of high infestation, at which point it might possibly be too late to favour colony survival.

These results suggest that selection of hygienic behaviour towards dead brood may not be adapted to select for resistance against *V. destructor* (Leclercq *et al*, 2017).

2.2.1.5 Post-capping duration

Studies concerning the post-capping duration have led to a majority of inconclusive results. One hypothesis explaining its lack of efficiency in providing resistance against *V. destructor* in selection programmes could be that, due to the physiological effects of parasitism, infested pupae experience a longer post-capping stage duration than non-parasitised pupae, which could cancel out any reduction generated by selection (Bienefeld and Zautke, 2007). Modelling approaches suggest that a decrease of two days in the post-capping stage duration of workers would be necessary to achieve a stable mite population over the years (Martin, 1998; Martin, 1997). Even if such a reduction were achieved after selection, it may result in more brood cycles per year, thereby likely counterbalancing the negative effect on individual mite reproduction by increasing the number of generations (Rosenkranz *et al*, 2010).

2.2.1.6 Grooming

A relatively high number of studies have shown no link between grooming and infestation levels (Table SF), which has decreased the interest of breeders in this trait (Aumeier, 2001; Bienefeld *et al*, 1999; Correa Marques *et al*, 2000; Kovačić *et al*, 2018; Rosenkranz *et al*, 1997). This finding may be due to the fact that observations of mite damage resulting from grooming can be ambiguous. Some deformations of the dorsal shield of the mite are related to the mite's ontogeny and so do not reflect the action of honey bee mandibles (Bienefeld *et al*, 1999; Davis, 2009; Lodesani *et al*, 1996). In addition, some damage can occur post-mortem (Bienefeld *et al*, 1999; Davis, 2009; Lodesani *et al*, 1996). Both factors lead to an overestimation of the effectiveness of grooming and an increase in the risk of recording false positives for this trait. Such an overestimation of the impact of grooming may also be linked to the fact that many initial studies were performed in controlled laboratory conditions, where the proportions of damaged mites obtained in caged bees differed from those obtained in field conditions (Hoffmann, 1993) due to the higher environmental variation seen in the field.

2.2.1.7 Infestation levels

Infestation levels, as they directly reflect the quantity of the mites present in the colony, seem to be the most direct measure for assessing the potential of a colony to survive. However, the parasite's population dynamics and its association with colony survival are not yet well understood. It is, therefore, difficult to identify the time at which representative measures can be acquired in order to obtain a good phenotype. The infestation rates of untreated surviving colonies can reach a peak during the summer or even in the spring, followed by a decrease that cannot easily be explained (Dettli, 2018). The profiles and timings of these peaks may vary among colonies, even within the same apiary, which makes it difficult to choose an optimal time point at which to record the infestation. Such colonies may either be tolerant to *V. destructor* or, perhaps, show a combination of tolerance and resistance, as has been shown in the cases of other organisms (Råberg *et al*, 2009). A colony with infestation rates considered to be above the survival threshold defined for susceptible populations might be excluded from a selection programme just ahead of its natural decrease in infestation, despite the colony being of interest for selection purposes. The methods commonly used by beekeepers to evaluate the infestation level may also show insufficient precision, for example, if they are performed on overly small worker samples or on a restricted number of colonies (Lee *et al*, 2010). In addition, these measures are biased by reinvasion as soon as workers rob collapsing colonies located in the neighbourhood and so vector mites back to their colony (section 2.2.2.3).

2.2.1.8 Conclusion

The traits described as conferring resistance to honey bee colonies may exhibit variable efficiency in terms of decreasing infestation rates, thereby limiting the efficiency of programmes selecting them. This limitation is mainly due to the compromises that have to be made between investing time and resources in developing theoretical knowledge about resistance and the need for solutions in the field. The prior research has been hampered by several limitations. As reliable field tests are difficult and time consuming to develop, mainly due to conditions that cannot be standardised, the results obtained from laboratory studies or small-scale field studies are generalised. However, even if laboratory conditions can generate promising results (Moretto *et al*, 1993; Moritz, 1985), they lack the robustness for direct translation to field conditions, especially when stocks with different genetic origins are used.

In addition, the selected traits were not linked to lower mite infestations in every population and, even if present, may not always be expressed at a sufficient level to have a direct impact on survival, leading to a correlation but not to a causal relation with survival. The choice of these traits stems from observations of surviving populations. However, a combination of mechanisms are thought to contribute to colony survival in several such populations (Locke, 2016). Grooming or VSH behaviours were expressed in several of them (Locke, 2016), although not in the Norwegian surviving population (Oddie *et al*, 2017). In addition, no differences between colonies from the Gotland population and susceptible controls were noticed in terms of hygienic behaviour, grooming or brood attractiveness (Locke and Fries, 2011), meaning that these resistance traits are unlikely to be part of the survival pattern of Gotland colonies. The presence of one resistance trait in one population, therefore, does not imply that the given trait favours survival in any given population. An *a priori* choice of resistance traits to select within susceptible populations is, thus, a hazardous strategy by which to obtain surviving colonies, as the chosen trait may not be linked to survival in the local environment.

More generally, a limited response to selection is expected when several traits interact to produce resistance or survival, although only one trait is selected. Increasing the frequency of a single trait to high levels so that no other trait is required might lead to resistance, but this may reach biological limits in the host: if workers are highly performing one resistance behaviour, they may have less time available to perform other tasks also essential to colony functioning. Selection for multiple resistance traits would be preferable, although such an approach is limited by our lack of understanding of the qualitative (which trait) and quantitative (level of expression of each trait) aspects of the combination required. Such a strategy would also be difficult to implement due to the higher workload required to evaluate several traits and then to select colonies for their joint expression.

2.2.2 Limitations due to environmental effects

Even when the selected putative resistance traits were linked to the colony infestation level or survival (section 1.1.1), their heritability was occasionally low (Table A), which indicated that their expression may be affected by environmental conditions. In this section, we present the environmental influences acting at landscape, apiary and colony levels that affect the expression of the resistance selection traits presented in Part I.

2.2.2.1 Climate

Environmental factors have been described to limit the accuracy of resistance traits. Among such factors, temperature and humidity are probably the most important, as they affect several traits. Significant between-year variations in the infestation growth rate between early and late seasons have been found to correlate negatively with temperature and positively with relative humidity (Harris *et al*, 2004). SMR has also shown between-year variation (Kulincevic *et al*, 1988), potentially due to temperature fluctuations affecting the number of viable offspring produced per female mite (Bienefeld *et al*, 1995). Similarly, grooming is affected by environmental factors (Pritchard, 2016), varying according to the season, with less grooming being performed in spring than in summer (Büchler, 1993). It has been shown that the impact of grooming on mite mortality is reduced at lower temperatures

(Currie and Tahmasbi, 2008; Tahmasbi, 2009) and at higher humidity (Tahmasbi, 2009), meaning that this trait in particular could be less efficient in terms of limiting the mite population during wintertime. Temperature effects detrimental to the use of the post-capping stage duration have also been recorded (Jay, 1963). Marked differences of up to 24 hours were observed in relation to this duration within individual colonies, possibly due to the heterogeneity of the brood temperature that drives brood development (Rosenkranz, 1990; Rosenkranz and Engels, 1994). This temperature impact results in strong seasonal effects regarding this trait: longer development times were measured in the late summer or fall when compared to the spring (Büchler and Drescher, 1990; Schousboe, 1990). The late summer and fall correspond to the crucial development of winter honey bees as well as to high mite infestations in untreated colonies: to have a protective effect, the post-capping stage duration should be kept short in selected colonies, even in the presence of lower ambient temperatures, which is physiologically unlikely.

2.2.2.2 Food resources

The expression of several traits also fluctuates according to the availability of food resources. This is particularly the case for hygienic behaviour, which is known to be expressed more when the food availability increased (Büchler, 1994a; Büchler, 1994b; Momot and Rothenbühler, 1971). Thus, repeated measures are required throughout the season to control for such variations and to obtain reliable values (Büchler, 1994a; Büchler, 1994b), which renders an efficacious selection programme based on this trait costly in terms of both time and resources. Similarly, it has been observed that rates of mite removal doubled after colonies were fed with sugar water (Boecking, 1994). The cells containing pupae could also be cleared by honey bees during times of pollen deprivation (Newton and Michl, 1974), leaving uncapped, cannibalised pupae difficult to distinguish from brood removed by VSH honey bees. Interestingly, it is not only the food quantity but also the quality that can affect selected traits: grooming was, for instance, found to decrease when workers were exposed to the pesticide clothianidin (Morfin *et al*, 2019b), which is periodically used in agriculture.

Depending on the year, seasonal events (climate, food resources) are, therefore, likely to explain low infestations to a greater extent than the potential response to the selection of specific resistance traits.

2.2.2.3 Beekeeping

The infestation level during the late summer and the infestation growth rate during the beekeeping season are both traits that could be expected to result from the initial infestation in the spring, the reproduction and longevity parameters of *V. destructor*, as well as the defense behaviour of *A. mellifera*. However, they are biased by the horizontal transmission of *V. destructor* between colonies, for example, due to drift or robbing (DeGrandi-Hoffman *et al*, 2017a; DeGrandi-Hoffman *et al*, 2017b; Frey and Rosenkranz, 2014; Frey *et al*, 2011; Greatti *et al*, 1992; Peck and Seeley, 2019; Ritter and Leclercq, 1987; Sakofski *et al*, 1990). Mite influx from neighbouring colonies and apiaries may not be visible to the evaluator and so might bias the phenotyping of selection traits. Unreliable results concerning the assessment of the resistance potential of a colony based on its infestation level are particularly likely to be generated if mite transfers differently affect colonies located within the same apiary. Indeed, the hypothesis underlying honey bee selection is that all colonies placed within the same apiary share identical environmental conditions. Unlike the case of sheep, where individual resistance levels can be accurately estimated even if the animals are kept in groups (Aguerre *et al*, 2018), the heterogeneity of horizontal mite transmission among honey bee colonies (Frey and Rosenkranz, 2014) is likely to render the impact of resistance traits on the infestation level less visible.

Horizontal transmission is more likely to occur in regions with high colony densities (Frey and Rosenkranz, 2014) or when the inter-colony distance within the apiary is low (Nolan and Delaplane, 2017; Seeley and Smith, 2015). In such situations, single colony resistance mechanisms with a significant influence on the infestation level may be more easily detected during periods featuring low horizontal mite transmission, for instance, during the early season or during honey flows. Removing ‘superspreaders’, that is, colonies above a certain infestation threshold (Råberg *et al*, 2009), could be an option for limiting horizontal transmission. This approach, however, entails the risk of removing potentially tolerant colonies, which are unharmed by elevated infestation rates or colonies which may start expressing resistance behaviour in the near future. In addition, infestation levels allowing for or compromising survival may also vary according to the mite-transferred virus types infecting the honey bee populations, as their virulence may have a direct effect on the number of mites that can be endured by the colony (Martin, 2001; Neumann *et al*, 2012). To date, our knowledge of *V. destructor* population dynamics and their relationship with colony survival is too fragmentary to allow the infestation rate to be an accurate selection criterion. Infestation thresholds critical to colony survival may vary according to environmental conditions and, therefore, do not offer suitable values for guiding selection programmes. Their effectiveness would be increased if they were locally determined, thereby avoiding as many biases as possible, although this would involve an extremely tedious process (Delaplane and Hood, 1997; Delaplane and Hood, 1999; Dietemann *et al*, 2013).

2.2.2.4 Brood production dynamics

The brood quantity directly influences infestation rates by defining the number of hosts available for mite reproduction. Fluctuations in brood production may at least partly explain why the within-colony distribution of *V. destructor* inside the brood is not spatially homogeneous (Fuchs, 1985; Wendel, 1989). These variations in brood production also affect the repeatability of VSH: a stronger VSH response will be obtained if many infested cells are clustered in a small brood area or are multiply infested (Cheruiyot *et al*, 2018; Kim *et al*, 2018). Thus, the phenotypic values can depend on the local infestation rate where the measurement is performed on the brood comb. The artificial infestation of individual cells may help to overcome such issues; however, this tedious technique may be difficult to implement in beekeeping conditions and so may be restricted to research programmes.

The proportion of damaged mites, which has been employed as a measure for estimating grooming behaviour, has also been shown to fluctuate with the presence or absence of brood and of young honey bees emerging. More damage was recorded when the brood was emerging (Hoffmann, 1995). This difference could be due to mites being more vulnerable to grooming when they are changing host from emerging bees to nurses (Lobb and Martin, 1997) when compared to mites adhering on adult workers in the absence of brood.

2.2.2.5 Other insects

Wax moths can bias the quantification of uncapping–recapping behaviour because the feeding activity of their larvae can also trigger honey bee nurses to open and close brood cells (Villegas and Villa, 2006). Wax moths also increase the risk of overestimating the percentage of damaged mites recorded on the bottom board (Bienefeld *et al*, 1999; Davis, 2009; Lodesani *et al*, 1996). To limit environmental biases in grooming measurements, it has been recommended to collect the fallen mites every two days (Bienefeld *et al*, 1999; Davis, 2009;

Lodesani *et al*, 1996), which increases the amount of time required for sampling. Ants can also bias the infestation level estimation stemming from natural mite fall by collecting fallen mites as a food resource (Dainat *et al*, 2011b), thereby limiting the accuracy of phenotypes when no measures are taken to limit the presence of ants next to the colonies (Dainat *et al*, 2011a).

2.2.2.6 Social environment

Social learning effects could also play a role as a resistance mechanism in honey bee colonies. Learning could be conveyed by communication between workers. Communication among honey bees allows for the transfer of information between individuals concerning many complex tasks, including nest site choice and the regulation of foraging (Laomettachit *et al*, 2016; Stabentheiner *et al*, 2010; von Frisch and Lindauer, 1993). Social learning has been identified in other insects, such as fruit flies (Danchin *et al*, 2018) and bumble bees (Avargues-Weber and Chittka, 2014; Baracchi *et al*, 2018; Worden and Papaj, 2005), which adapt their behaviour after observing conspecifics. It has rarely been investigated in honey bees though, which is perhaps surprising given that they are a model organism in which to study learning. The social transmission of hygienic behaviour towards dead brood was experimentally ruled out (Trump *et al*, 1967), although it could play a role in other resistance traits against *V. destructor*.

2.2.2.7 Conclusion

The resistance traits described in the literature seem to have an effect on mite loads in some populations (section 1.1.1). However, they can be highly influenced by environmental conditions (Figure 1). In order to choose appropriate traits for resistance selection in a defined region, the calculation of heritabilities is necessary to determine which traits have a detectable genetic component and can, therefore, be selected. As long as the impact of the local environment on the ability of the selected traits to limit infestation remains unknown, progress towards surviving stock is likely to be limited. Greater knowledge of environmental effects on colony survival is required to better design and increase the efficiency of selection programmes. This requires resource-intensive research, which has, to date, not been performed.

2.3 Non-heritable survival factors

The evaluation of the resistance traits of colonies is fraught with numerous difficulties (section 2.2.2.7), and selection for these traits does not guarantee colony survival (section 2.2.1.8). In section 2.2.2, the environmental factors that limit the expression of resistance traits have been presented. It may, therefore, appear easier to select for colony survival by means of natural selection. However, doing so may result in colonies whose survival is not due to a higher **genetic value**, but rather to particular environmental conditions (Figure 1). Hence, these colonies may not constitute a suitable population for breeding: they may not survive if moved to a new location or if the local environmental conditions change. In this section, we present the environmental factors that impact colony survival.

2.3.1 Local *Varroa* haplotype and virus strain

In some cases, the survival of *A. mellifera* could be linked to less virulent virus populations being vectored by the mite, which would result in colonies tolerating more mites. Tolerance was, for instance, observed in surviving *A. m. ligustica* colonies from the isolated population of the island of Fernando de Noronha, Brazil, which have survived high *V. destructor* infestations for many years (de Jong and Soares, 1997; de Mattos *et al*, 2016). This survival may be linked to the lower virulence of the Japanese haplotype of *V. destructor* found on the island (Strapazzon *et al*, 2009) or of the carried viruses (Brettell and Martin, 2017) and not to the active resistance behaviour of the host. Indeed, colonies re-located from this origin that have been exposed to different mites (Korean haplotype of *V. destructor)* and different virus types did not show higher survival than the local susceptible controls (Correa Marques *et al*, 2002). The survival of the Gotland population was also attributed, in part at least, to a tolerance against viral infections (Locke, 2016; Locke *et al*, 2014; Thaduri *et al*, 2018; Thaduri *et al*, 2019). In Wales (Table SH), the involvement of viruses in colony survival is hypothesised via the superinfection exclusion of the virulent variant DWV-A by the less virulent variant DWV-B (Barroso-Arévalo *et al*, 2019b; Mordecai *et al*, 2016). However, other results suggest the higher virulence of DWV-B when compared to DWV-A (Gisder *et al*, 2018; McMahon *et al*, 2016). The contribution of the virus populations to the observed survival of these colonies, therefore, remains unclear. Viral loads also fluctuate during beekeeping season (Dainat *et al*, 2012a; de Miranda *et al*, 2011; Desai and Currie, 2016; Tentcheva *et al*, 2004) or following colony migration (Alger *et al*, 2018), which directly impacts the virulence of the mites. To improve selection programmes, a better understanding of *V. destructor* virulence and its link to the dynamics and evolution of virus populations is required.

2.3.2 Nesting habits

A key problem associated with natural selection is that it leads to an ambiguous result, as not all surviving colonies may express active defensive behaviour against *V. destructor*. Some may only take advantage of a favourable habitat or low honey bee density, and it is likely that they would not survive if environmental changes occurred. Several cases of probably non-resistant yet surviving colonies have been documented: for example, in the Arnot Forest (New York, USA), a small population of feral colonies of European *A. mellifera* origin survives without treatments or care by a beekeeper. The colonies nest in small cavities, for instance, in hollow trees. In a comparison with control colonies, the colonies bred from the feral origin did not show reduced infestation levels (Seeley, 2007). Therefore, their persistence may not be related to genetic host resistance, but rather to limited horizontal transmission in the context of low colony density (Seeley, 2007). In addition, the small volumes of tree cavities limit the production of brood, promote frequent swarming and increase air humidity, which are all putative factors limiting the development of high infestation levels (Loftus *et al*, 2016; Mitchell, 2019). Comparable survival rates among non-resistant colonies were obtained with unmanaged hived colonies of the same size (Seeley, 2017b), thereby confirming that survival can be promoted by non-genetic factors.

Feral colonies are thought to occur in many regions (Bargain, 2018; Kohl and Rutschmann, 2018; Oleksa *et al*, 2013; Requier *et al*, 2019), although it is unclear how long such colonies survive. When these colonies survive for more than two or three years, it is not known whether they express genetic resistance against the parasite or whether they survive solely due to favourable environmental conditions. The presence of feral colonies that survive due to non-genetic characteristics may have detrimental effects on selection programmes for resistance in the beekeeping context if they are present in the mating area (Erickson *et al*, 2002). Their sympatry is likely to lead to admixture with non-resistant alleles and, therefore, to an important decrease in the selection progress (Plate *et al*, 2019). Although non-resistant surviving colonies are of little use for selection purposes, their study could inspire new management strategies for managed colonies, as promoted by the 'Darwinian' beekeeping (Neumann and Blacquière, 2017; Seeley, 2017a).

2.3.3 Genotype–environment interactions

Several populations of the western honey bee previously described as showing resistance have been used as genetic donors to be introduced into selection programmes led by beekeepers or honey bee research institutes. The colonies headed by imported queens often failed to show better resistance or survival than the controls in their new environment. This is likely to be linked to genotype–environment interactions (Figure 1), which have been shown to favour the survival of locally selected colonies in untreated conditions (Büchler *et al*, 2014; Meixner *et al*, 2015). Low survival was, for example, observed for naturally surviving colonies imported from Uruguay to France, Poland and Germany (Koeniger *et al*, 1995; Rosenkranz, 1999; Ruttner *et al*, 1984), for local colonies imported from Brazil to Germany (Correa Marques *et al*, 2002) and for offspring of *A. m. capensis* imported from South Africa to Europe and crossed with *A. m. carnica* (Wilde and Koeniger, 1992). Similarly, colonies from the Avignon surviving population did not show differences in infestation levels or survival rates when compared to local stock when moved to foreign European countries (Locke, 2016; Meixner *et al*, 2014; Meixner *et al*, 2015) or to Canada (Vaublanc *et al*, 2003). Queens from the 'Russian' lineage imported from the USA to Germany and tested as pure mated queens or F1 hybrids (Rosenkranz, 2003) showed lower infestation rates and more damaged mites on the bottom boards than the local susceptible controls (Berg *et al*, 2001b). However, this could be explained by the lower colony and brood sizes seen in the 'Russian' lineage (Rosenkranz and Liebig, 2003; Schuster, 2003). The low survival of the 'Russian' colonies was also observed (Rosenkranz and Liebig, 2003). It is likely that the low resistance measured in Germany is due to a lack of adaptation to the new environmental conditions.

These results indicate that the potentially high influence of adaptation to local conditions on the ability to survive may restrain the suitability of naturally selected lineages for beekeepers located in, or migrating their colonies to, regions with different environments, as well as for beekeepers located in regions with highly fluctuating environmental conditions. In the current context of increased climatic variations (Vasseur *et al*, 2014) having direct impacts on both plants and pollinators (Martin *et al*, 2019a; Phillips *et al*, 2018), it is likely that, in the future, survival due to adaptation to environmental conditions may be achieved less frequently than in the past. Colony survival due to heritable traits should, therefore, be preferred. To conclude, despite the existence of a few apparently successful examples (see

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section 1.2.2), attempts to import surviving material from other regions or environments should not be supported. Aside from the uncertain performance of the introduced stock, detrimental side effects such as admixture with local populations and the risk of introducing foreign pests and pathogens have to be taken into account (Owen, 2017). As there is no guarantee that survival is a heritable trait, taking advantage of genotype–environment interactions by working towards surviving stock within groups of beekeepers operating in similar environmental conditions is recommended (Figure 1). The difficulty here involves determining which environmental factors are important and identifying the regions in which they are similar.

The examples presented in this section indicate that surviving populations can be obtained by means of natural selection (see also Table SH); however, the heritabilities of survival have not been calculated and, further, are likely to be low due to high environmental influences. As a result, natural selection should be only initiated on part of the population in order to limit the risk of losing all colonies if no resistance traits enabling survival are present or if the local ecological niche does not allow for the survival of non-resistant colonies.

2.4 Limitations of the implementation of selection strategies in beekeeping practice

Honey bee lines capable of surviving *V. destructor* infestation originating from selective breeding or natural selection would be of little interest for beekeeping purposes if they did not express other desirable traits (section 2.4.1) or if they could not be conserved due to the high workload required (section 2.4.2).

2.4.1 Suitability of selected stock for beekeepers

Selected stocks have to meet the expectations of beekeepers if they are to be adopted. They need to efficiently (in terms of both money and honey) protect the colonies against *V. destructor* despite the high horizontal transmission of the parasite in high-density apiaries (Frey *et al*, 2011; Fries and Camazine, 2001; Seeley and Smith, 2015). As horizontal transfer occurs beyond the apiary (Frey *et al*, 2011), resistant lines would also need to be used by all beekeepers in a given region so as to limit the negative impact of mite pressure from neighbouring apiaries, which could override resistance abilities. The extent to which resistance to *V. destructor* can be promoted is also likely to be limited by other desirable traits that are part of the selection objectives.

Surviving colonies originating from natural selection may not be suitable for all beekeeping operations. Indeed, high productivity, low defensive behaviour, behaviour on comb and a low propensity to swarm are often desired by beekeepers, although they are not necessarily favoured by natural selection (Neumann and Blacquière, 2017). To date, the surviving populations from Gotland and Avignon have not been multiplied for commercial beekeeping, most likely due to the small colony size (Locke and Fries, 2011), limited honey production (Le Conte *et al*, 2007; Locke, 2016), high propensity for swarming (Fries *et al*, 2006) and high defensive behaviour (Locke, 2016). Similarly, Africanised honey bees express resistance against *V. destructor* (Calderon *et al*, 2010; Martin and Medina, 2004; Schneider *et al*, 2004), but also detrimental traits, such as marked defensive and swarming behaviours. Their spread caused a decrease in apicultural activity in South and Central America, and they only became profitable for beekeeping purposes following a significant adaptation of management practices (De Jong, 1996; Francoy *et al*, 2009; Ratnieks and Visscher, 1996; Tibatá *et al*, 2018). The 'Russian' stock tested in Germany revealed lower honey production, smaller population size, lower calmness during inspection and higher defensive behaviour than the local controls (Berg *et al*, 2003; Boecking and Schönberger, 2003; Rosenkranz and Liebig, 2003; Schuster, 2003):. As a result, the import of this stock was not recommended for German beekeepers (Rosenkranz, 2003).

In addition, detrimental traits could also appear in colonies selectively bred for resistance to *V. destructor*. A study conducted in Austrian *A. m. carnica* colonies found a positive phenotypic correlation (r = +0.17) between the infestation level and honey production. Despite the low coefficient of the correlation, this observation implies that colonies producing more honey also reared more brood, thereby promoting mite reproduction (Boigenzahn and Willam, 1999). Such trade-offs may also occur in some lineages selected for VSH, which showed poor brood patterns (Rinderer *et al*, 2010) and may express suboptimal colony development, which would hinder honey collection or pollination ability. Similarly, despite artificially uncapped/recapped worker brood being found to have similar adult longevity when compared to controls (Siceanu, 1997), negative effects on their behaviour and performance could not be excluded (Harris *et al*, 2010). A possible trade-off was also observed between SMR and honey production. Colonies selected for SMR were found to be smaller than the controls or hybrids (Delaplane *et al*, 2005; Harbo and Harris, 2001). In a German population selected for hygienic behaviour and grooming when compared to control colonies in various environments, the lower colony size, lower gentleness and slightly lower spring honey harvest of the selected colonies were recorded (Büchler, 1997a; Büchler, 1997b).

The extent to which decreased performance may hinder the acceptance of a resistant stock by beekeepers is currently only poorly understood. Among a group of Swiss beekeepers who were mainly operating as hobbyists, a survey of priorities in terms of desired traits revealed that honey bees resistant to *V. destructor* would be favoured even if they expressed other detrimental traits (Guichard *et al*, 2019), indicating that resistant stock would be more easily accepted by beekeepers with a low, or no, profitability goal. As the interest of beekeepers in a selected stock is of principal importance when it comes to favouring the adoption of resistant lines or the establishment of new surviving populations, surveys (Guichard *et al*, 2019; Leiby, 2014) and marketing analyses could help to define currently valued traits before initiating selection programmes. A further challenge here concerns the fact that the desired traits might change faster than the corresponding lineages can be generated by means of selection, which may, at best, take several years.

2.4.2 Field evaluation of honey bee resistance traits

An important limitation concerning the implementation of resistance traits in the field is the fact that their evaluation is tedious. Selecting for SMR or VSH is very time consuming, as a minimal number of infested brood cells need to be found in order to generate reliable results (Büchler *et al*, 2017; Dietemann *et al*, 2013). Reaching this number may require the opening of several hundred cells. The lower the infestation rate, the higher the number of cells that would need to be opened. The crux of the matter is that the most interesting colonies for selection, that is, those with the lowest amount of mites, are the most time consuming to evaluate. To work around this limitation, the desired number of cells could be manually infested, although this would also prove time consuming and would require the maintaining of highly infested colonies as mite donors (Dietemann *et al*, 2013). It is, therefore, only doable for a small number of colonies, in research programmes or in large cooperating networks able to provide a sufficient workforce (e.g. Arista Bee Research Foundation, www.aristabeeresearch.org).

In addition, several traits were found to require repeated measurements to generate reliable estimates (Büchler, 1994b; Villa *et al*, 2017). For some traits, such as infestation levels, this is due to the fact that late values cannot be predicted from values obtained early in the season (Rademacher, 1985). Under field conditions, many variations in infestation levels throughout the year are not detected: currently, in Europe, beekeepers from testing networks generally take one or two snapshot measurements of the infestation rate during the spring or summer. This may prove insufficient to detect the particular infestation patterns of colonies self-regulating their infestation rate once a certain infestation threshold is reached. The continuous evaluation of the infestation rates of the best selection candidates may help to gain additional information regarding their resistance potential, although it would be very time consuming. Automatic methods, for example, image-based analysis, would be required to circumvent this limitation by decreasing the workload for the beekeeper.

Natural selection involves a lower workload than selective breeding, as it does not require the estimation of phenotypes. However, as it is likely to lead to high colony losses, at least during the first few years of the programme (section 1.2), it may suffer from poor acceptance, especially among beekeepers who make a living from their activity (Kefuss *et al*, 2016).

III. Perspectives on sustainable progress towards surviving stocks

Numerous limitations that could potentially hinder the progress of selection have been presented in Part II. Using the knowledge of *V. destructor* resistance selection acquired over three decades, we describe, in the following sections, a practical strategy for maximising the positive outcomes of selection programmes for increased survival against *V. destructor* infestations. Short-term optimisations of selection programmes are presented in section 3.1, while elements to be considered in relation to the long-term stewardship and sustainability of these programmes are suggested in section 3.2.

3.1 Improving selection strategies

In this section, we present a strategy for increasing the efficacy of selection for enhanced colony survival. It is based on the establishment of an untreated test population for the identification of local resistance traits (section 3.1.1) as well as on selection for this trait in related stock managed by beekeepers in the same area (section 3.1.2). Genetic progress can be optimised by improving management of the stock (section 3.1.3), while costs related to phenotyping could be decreased in the future through the integration of genomic data (section 3.1.4). Encouraging partnerships among beekeepers and researchers (section 3.1.5) could facilitate the development of new techniques and strategies in the field.

3.1.1 Identifying local resistance traits

The success of selective breeding programmes for honey bee resistance against *V. destructor* depends on the characteristics of the chosen traits (high heritability, hypothesised strong correlation with survival) and on the accuracy of trait phenotyping. If a trait is at least partly of genetic origin and correlates with better colony survival, it should be suitable for selection in different environments. The use of **pedigree** information together with colony performance data should allow for the yearly calculation of breeding values and the estimation of genetic progress for each selected population, thereby providing useful monitoring tools.

Based on these considerations, for regions where no surviving colonies are naturally present, we propose a procedure for increasing the success of selection by locally selecting for resistance traits (Figure 2). The identification of these traits could be performed in a dedicated untreated stock (test population). This test population could be established from a subset of the genetic material reared by local beekeepers (beekeeping population) or from material that has survived for at least a few years (Figure 2, ❶).

Maintaining an untreated test population is, however, an uncertain process: the outcome of the survival experiment may be highly dependent on local environmental conditions and on the number of colonies expressing resistance traits that are already present in the population, so the probability of success cannot be predicted. During the first few years following the creation of the test population, high colony losses are likely to occur (Fries *et al*, 2006; Kefuss *et al*, 2016). If resistance traits are not present in the population, or if they are present but at too low a level or too low a frequency, the entire tested stock is condemned (Berg *et al*, 2001a; Blacquière *et al*, 2020). In addition, it should be noted that deliberately inducing the death of colonies is considered to be unethical and contrary to animal welfare standards (World Organisation for Animal Health, 2018). It is, therefore, forbidden in some countries, for example, Germany (Bundesministerium der Justiz und für Verbraucherschutz and Bundesamt für Justiz, 2014) and Switzerland (Conseil fédéral suisse, 1995). Removing colonies that are showing signs of failure from the test population prior to their collapse, treating them and then reintegrating them into the beekeeping population (the unselected stock under routine management, including varroacide treatments) could limit colony deaths.

Untreated test populations should be hosted at isolated locations. This will reduce mite transfers from untreated colonies (Büchler and Hoffmann, 1991) to neighbouring apiaries not participating in the programme. Isolation will also favour closed-population mating and, thus, the response to selection enabling adaptation to local conditions (Szabo and Lefkovitch, 1987). Mating stations on islands or in remote mountain valleys, as well as artificial insemination (Page and Laidlaw, 1985), offer suitable possibilities to avoid introgression with unselected populations.

3.1.2 Assessing trait heritability and selecting

To maintain a broader genetic background for selection than simply that of the test population, colonies from the beekeeping population showing high values for traits linked to survival in the test population can then be selected: this leads to the creation of a selection core from the beekeeping population (Figure 2, ❷). The advantage of this approach when compared to using only the test population as a genetic resource for queen rearing is that this selection for resistance traits can be performed under standard beekeeping conditions, on a broader scale and with limited risks of colony losses. The selection process could occur within a regional network of apiaries in order to maximise genetic diversity within the selection core and decrease the risk of failure for the individual beekeeper. To evaluate the effectiveness of selection in terms of colony survival, the selection core should be maintained with minimal acaricide treatments. The local heritability for the selected trait should be estimated in this core from recordings over a few generations. Depending on the population structure, several hundred colonies need to be included in the analysis so as to provide sufficiently reliable estimates. Simultaneously, breeding values should be calculated and their evolution compared with a reference population to allow for the quantification of genetic progress in the selection core. Progress could also be quantified using survival tests or measurements of infestation levels in comparison to proven susceptible controls. A method for ensuring the susceptibility of such controls could be to choose colonies requeened with queens taken from untreated collapsing colonies. The correlation between the selected trait and the observed mortality (in untreated conditions) or treatment frequency (if colonies are still treated) should also be assessed to confirm that the trait (or traits) selected does indeed lead to the breeding goal. If no heritable traits are linked to lower mortality, it could be due to environmental effects (section 2.3) and so breeders could limit such effects to increase the probability of detecting resistance variations of genetic origin. This could, for instance, be achieved by installing apiaries in areas with low colony density, by removing too highly infested colonies from the apiary, by limiting drift and robbing situations by adapting the apiary or hive entrance design, or by adopting local good beekeeping practices.

Once genetic progress is achieved in terms of improving colony survival within the selection core, the latter can be used as a genetic resource for breeding by beekeepers (Figure 2, ❸). Rather than replacing the whole beekeeping population with the offspring of the core population, the latter should be used to introgress survival-linked alleles into the beekeeping population: this will favour the maintenance of maximum genetic diversity within the beekeeping population (section 3.2.1).

3.1.3 Optimising genetic progress

Selection based on heritable traits is not the only condition for obtaining genetic progress. Following the breeder's equation (Falconer, 1960; Lynch and Walsh, 1998), the response to selection is also a function of the selection intensity, the precision of the selection and the generation interval. Optimising these parameters would help to increase the response to selection within the selection core (Figure 2, ❷), especially if high heritabilities were estimated but no genetic progress was observed.

To increase the selection intensity for resistance traits, several measures can be taken. An **index selection** can be used to include both resistance-related and other traits into the breeding goal, thereby limiting possible tendencies to finally select based only on other traits than resistance, even when resistance traits were assessed. Calculating the genetic or phenotypic correlations between traits can help with anticipating the adverse effects of selection for resistance traits on the expression of other traits. In addition, the negative impacts of queen mortality, which limit the number of selection candidates and the selection differential, on selection intensity can be mitigated through management practices. Marking queens with coloured tags (Human *et al*, 2013) assists with their localisation, which limits the accidental loss of the queen. Keeping only strong colonies that are able to survive winter and limiting the pathogen spread through good beekeeping practices promote the maintenance of the selected lines.

The precision of the measurement required to obtain reliable breeding values could be achieved by promoting the correct use of accurate and standardised evaluation protocols during training courses. Protocols should be designed to record inter-colony variation at each test apiary and to enable the ranking of all colonies. To monitor the phenotypic variation of subjective traits (e.g. defensive behaviour), non-defined relative ranking systems should be preferred over pre-defined scales (Zakour and Bienefeld, 2013). Setting up test apiaries with 12–20 colonies facilitates ranking and standardises environmental conditions, as measurements of such a low number of colonies can be performed within a short period of time.

Early predictors of colony survival or infestation are preferred as selection traits for decreasing the generation interval and accelerating selection. It is, however, unclear whether increased research effort could help in identifying a reliable early predictor of late season infestation levels, or whether the environmental effects on the *V. destructor* population dynamic are too important. The potential for shortening the generation interval is also limited by the need to repeat measurements in order to obtain accurate phenotypes. Nonetheless, it is recommended to prioritise decreasing the generation interval so as to maximise genetic progress. Testing colonies during two subsequent years decreased the selection progress by ~40% when compared to a one-year testing approach (Bienefeld, 2003). In addition, long generation intervals increase the risk of queen losses, thereby potentially decreasing the selection intensity.

3.1.4 Integrating genomic data

The inclusion of genomic data in selection programmes could limit the workload associated with trait recording in the selection core (Figure 2, ❷). Genomic methods allow for the screening of a large number of colonies for sequences associated with known resistance traits or survival. This widespread monitoring may help to identify numerous loci with a direct impact on the resistance of colonies of western honey bee against the *Varroa* mite. Many loci linked to diverse resistance traits have already been identified (Arechavaleta-Velasco *et al*, 2012; Behrens *et al*, 2011; Broeckx *et al*, 2019; Guarna *et al*, 2017; Hamiduzzaman *et al*, 2017; Harpur *et al*, 2019; Hu *et al*, 2016; Jiang *et al*, 2016; Lapidge *et al*, 2002; Morfin *et al*, 2019a; Oxley *et al*, 2010; Spötter *et al*, 2016; Spötter *et al*, 2012; Tsuruda *et al*, 2012; Zakar *et al*, 2014). Even though each of these studies mentions the possibility of using marker-assisted or genomic selection in honey bee breeding, to the best of our knowledge, the identified sequences have not been routinely implemented in selection programmes. This might be because no single trait has been proven to increase colony survival on a broad scale, which could potentially limit the implementation of genomic selection to sufficiently wide populations to render it cost effective. A single field result linked to the implementation of genotyping tools in selection programmes has been reported to date (Guarna *et al*, 2017). An increase in hygienic behaviour and better survival against *V. destructor* were reported for colonies selected based on the presence of proteins linked to hygienic behaviour against freeze-killed brood when compared to unselected controls (Guarna *et al*, 2017). Colonies selected based on the identified markers exhibited similar survival to those selected based on their phenotypes (Guarna *et al*, 2017), indicating that genotyping can be as powerful as phenotyping when it comes to achieving progress with resistance selection. Several sequences associated with hygienic behaviour were identified and found to be predictive for this trait in unselected populations (Harpur *et al*, 2019), suggesting that marker-assisted selection could be extended to more colonies and, possibly, different populations. Survival in the absence of treatments was only tested over one year (Guarna *et al*, 2017), meaning that the findings require further confirmation through monitoring the following generations.

Even when marker-assisted or genomic selection programmes would facilitate selection, the phenotypes or, at least, survival would need to be evaluated in a representative part of the population so as to adjust the sequences used and to evaluate the selection progress. Genomic selection should, therefore, be considered as a means of selecting animals with increased precision (by considering their genomic data in addition to their phenotypes and pedigree) rather than as a tool for replacing trait evaluation.

3.1.5 Encouraging partnerships

As traits linked to resistance are time consuming and costly to evaluate, they may be only poorly implemented in beekeeping selection programmes. Support should be provided to beekeepers by research institutes, specialised breeding institutes or extension services. Mutually beneficial situations could be fostered through beekeeper–research partnerships: beekeepers' associations could own the colonies and multiply the stocks, while expert teams could provide evaluation protocols, perform certain measurements, estimate genetic parameters and genetic progress, and manage datasets or train beekeepers to perform such tasks. An advantage of this approach would be that research institutes would have regular access to large datasets without the need to maintain their own large stock for studies on traits. Large honey bee populations with pedigree information would also facilitate the implementation of genomic selection, as several thousand individuals need to be evaluated and genotyped following this approach. In turn, beekeepers could benefit from the genetic progress and knowledge transmitted by experts and scientists. Beekeeper–research partnerships (e.g. CSI Pollen COLOSS, Bee Informed Partnership) and international experiments (e.g. COLOSS Genotype-Environment interaction experiment (Costa *et al*, 2012)), as well as collaboration between scientists, as performed within the frame of the COLOSS network (www.coloss.org) or the International Honey Bee Breeding Network (www.ihbbn.org), could favour the exchange of knowledge concerning selection procedures and enable the evaluation of selected stock in a wide range of environments.

3.2 Maintaining the acquired host–parasite balance

To ensure the sustainability of successfully selected lineages, evolutionary processes need to be taken into account. According to the 'Red Queen' hypothesis (van Valen, 1973), host–parasite relationships are governed by an arms race between the involved species. Therefore, even if honey bee populations resistant to their parasite were obtained, a risk exists that mites could acquire the capacity to bypass the selected honey bee defense behaviours in the same way that mites have acquired resistance to acaricides in several regions (Elzen *et al*, 1999; González-Cabrera *et al*, 2018; González-Cabrera *et al*, 2016; Hubert *et al*, 2014; Lodesani *et al*, 1995; Stara *et al*, 2018; Trouiller, 1998). Advantageous new chemosensorial capacities or modified reproductive cycles could be naturally selected in mites and so represent selective advantages for adapting to the complex chemical cues emitted by honey bees (Nazzi and Le Conte, 2016). Such a phenomenon could, for instance, limit the efficacy of resistance traits linked to mite reproduction. Hence, sustaining the fitness of the host (section 3.2.1) and limiting the increased virulence of the parasite (section 3.2.2) should be favoured in the long term in selected populations in order to secure future gains in selection towards achieving host–parasite equilibrium.

3.2.1. Maintaining the genetic diversity and fitness of the host

Genetic diversity within honey bee colonies directly contributes to their fitness: within-colony genetic diversity is, for instance, likely to contribute to limiting *V. destructor* infestation (Delaplane *et al*, 2015; Desai and Currie, 2015; Schmid-Hempel and Koella, 1994). Conversely, inbreeding is known to have detrimental consequences for honey bee health and fitness (Brückner, 1979; Moritz, 1986; Tarpy *et al*, 2013; Woyke, 1976).

Selection performed with a high intensity could rapidly decrease the genetic diversity and limit the resilience and long-term survival of a population. Alleles that could be adaptive in the future against new pests or pathogens could be lost if selection focuses solely on resistance against the current invasive lineage of *V. destructor*. Genetic losses linked to choices when pursuing selection objectives have been reported in plants, in which resistance to herbivory was found to be less expressed in domesticated than in wild genotypes (Chaudhary, 2013; Soltis *et al*, 2019). This difference was likely due to the focus of the selection on palatability, that is, against the production of bitter anti-nutritional compounds that deter herbivory (e.g. Enneking and Wink, 1999). The negative effects of selection on genetic diversity are magnified in small populations. Such populations are also exposed to genetic drift, which could have stronger effects than selection (Page and Laidlaw, 1982). Further, it could eliminate favourable resistance alleles and limit the capacity of the population to face future challenges. Genetic drift and the loss of resilience may occur in the Gotland population, which went through a strong bottleneck following selective pressure by *V. destructor* (Lattorff *et al*, 2015),

Losses in genetic diversity could be counterbalanced by the polyandrous mating system of honey bees (Mikheyev *et al*, 2015). However, the resulting gain in diversity would be constrained by the size and genetic diversity of the remaining population as well as by the occurrence of beneficial recombinations, which is unpredictable. In addition, rare beneficial genes (Fuchs and Moritz 1998) might be permanently lost. As selection cannot be performed in a proactive manner, limiting genetic erosion (Bellon *et al*, 2017; Peres, 2016; Van De Wouw *et al*, 2010) and maintaining genetic resources less favourable for production as a genetic backup are recommended for increasing the resilience potential against future threats in a variety of crop and livestock species (Brush, 2000; Notter, 1999). This is also desirable in the case of honey bees (Parejo *et al*, 2016), wherein such traits could help when facing new parasites. The risk of allele losses, either due to selective breeding or genetic drift, could be avoided by maintaining colonies of the same lines as those included in the untreated test population and the selection core under normal *V. destructor* management as a genetic back-up. The genetic diversity of the selection core can, therefore, be increased if required by introducing a few back-up colonies to the core each year so as to add supplementary alleles.

3.2.2. Reducing the genetic diversity and virulence of the parasite

The efficiency of host resistance traits may vary according to the genome of the parasite (Garrido *et al*, 2003; Morgan and Koskella, 2017). Natural selection in mites infesting managed selected colonies could lead to selective trajectories impacting both the genetic diversity and virulence of mites (Dynes *et al*, 2019; Eliash and Mikheyev, 2019; Techer *et al*, 2019), thereby possibly limiting the efficiency of previously selected traits against the parasite. As more *V. destructor* haplotypes, as well as *Varroa jacobsoni* and *Varroa underwoodi*, may shift towards *A. mellifera* (Beaurepaire *et al*, 2015; Navajas *et al*, 2010; Roberts *et al*, 2015; Wang *et al*, 2019), it is unknown whether defence traits against the currently widespread invasive *V. destructor* Korean haplotype would also prove effective against other mite lineages or species.

If the detrimental effects of mites that are not already present is difficult, if not impossible, to anticipate, reaching a sustainable host–parasite relationship between current local mites and their host should be the first objective. This can be facilitated by exploiting the principles of evolution of virulence (Cressler *et al*, 2016; Read, 1994) in apicultural practices in order to limit natural selection for more virulent mites. In apiaries in which many colonies are kept within a limited space, parasites killing colonies during the late summer or early autumn have a selective advantage, as the collapse of the host colonies enables the horizontal transmission of mites by robbers both within and between apiaries (Peck and Seeley, 2019; Seeley and Smith, 2015). Common beekeeping practices also include the constant replacement of dead colonies; thus, the pressure to achieve a decrease in parasite virulence is relieved and, instead, its increase is promoted (Fries and Camazine, 2001). Simple interventions, such as removing or treating highly infested colonies before their death, may not only improve the survival of neighbouring colonies by decreasing the horizontal transfer of mites, but also limit the selective pressure on the parasite. Adapting the apiary layout to reduce drift between colonies (Jay, 1966a; Jay, 1966b; Jay, 1968; Nolan and Delaplane, 2017) and adapting beekeeping practices to ecological conditions favourable to the honey bee should be favoured (Blacquière and Panziera, 2018; Brosi *et al*, 2017; Seeley, 2017a) in order to promote host fitness and limit the natural selection of virulence in the parasite.

Aside from horizontal transmission, the hybridisation of parasites can generate new genotypes with increased virulence (Dietemann *et al*, 2019; Greenspan *et al*, 2018; King *et al*, 2015). In *V. destructor*, recombination between genomes occurs among the offspring of different foundresses in multiply infested cells. The frequency of these cells increases with the infestation rates of colonies (Beaurepaire *et al*, 2017a); therefore, an increase in the genetic diversity of mites at the end of the beekeeping season occurs (Beaurepaire *et al*, 2017b) and creates variability, which could provide the parasite with advantages in the host–parasite arms race. The treatment or removal of highly infested colonies is, therefore, also useful in terms of preventing recombination events.

The virulence of mites is largely influenced by the viruses they vector. For instance, DWV is known to extend the honey bee development time (Koziy *et al*, 2019), thereby providing more time for the mite to perform reproduction. In addition, viral sequences are rearranged when DWV changes host, likely increasing its virulence (Gisder *et al*, 2018). The selected resistance traits may not remain efficient with regards to protecting colonies if the viruses associated with the mite became more harmful, as this would reduce the infestation threshold for colony collapse. However, highly virulent viruses, for instance, those causing the death of the pupae or limiting the fitness of the mite (Giuffre *et al*, 2019), would also be excluded by natural selection. This explains why the most common *V. destructor*-associated viruses infecting honey bees are of relatively low virulence (Martin, 2001; Sumpter and Martin, 2004). Mite–honey bee, mite–virus and virus–honey bee relations all, therefore, affect colony fitness, and greater knowledge of these interactions is required to secure sustainable host–parasite relationships.

3.3 Publishing the results of selection programmes

During the preparation of this review, we noticed that much information regarding selection programmes was either missing or else not readily available, especially for directed selection programmes. This lack of information constitutes a major limiting factor when it comes to achieving progress towards the successful breeding of resistant populations. The lack may be because selection programmes are often organised by beekeepers, who may have no interest in publishing their experiences and results, especially not in scientific journals.

Occasionally, the results of selection programmes or experiments were published in beekeeping journals or presented at beekeeping conferences, but not often in the English language, which limited their impact.

Among the publications we found, many reported promising results during the first few years of selection programmes, although none reported long-term success. There is also no information as to why particular programmes were discontinued, for example, whether due to insufficient success or other reasons. The field is, therefore, likely suffering from a publication bias, as positive and encouraging results are more frequently published than negative ones. The lack of information concerning failed initiatives is harmful for both the choice of traits and the design of future selection programmes. It may lead to a loss of resources and time when past mistakes are repeated. Reviewing the literature also highlighted significant variability in the definition of terms, such as resistance, tolerance, VSH, SMR and hygienic behaviour, resulting in ambiguous information. More attention should be paid to the clear definition of the recorded traits so as to avoid confusion. Both ahead of and during the publication process, attention should also be paid to the avoidance of methodological and reporting shortcomings. The calculation of breeding values for resistance against *V. destructor* is often either not performed or else the precise calculation method, selection progress and correlations between traits are not mentioned. The calculation methods are also often missing for heritability estimations, leaving the results difficult to compare and interpret. Mentioning the evolution of phenotypes over time is not sufficient to indicate genetic progress, as phenotypes may vary due to yearly effects of environmental origin. In practice, many programmes do not perform these controls (Cakmak and Fuchs, 2013; Szabo and Szabo, 2003) or else they do not publish the relevant data, meaning that they offer no proof of genetic progress due to selection.

In conclusion, we appeal to breeders to publish unsatisfying as well as satisfying selection results, and of the latter, cases in which the traits were not heritable are of particular importance. Providing heritability estimates (together with their calculation methods), even if they are null, would generate helpful knowledge for guiding the choice of selection traits.

Conclusions

We found no evidence that the selection strategies performed over the last few decades, whether relying on selective breeding or natural selection, resulted in large-scale solutions for tackling the detrimental effects of *V. destructor*. Several factors can explain the lack of widespread availability of selected lineages capable of surviving infestation by this parasite. Selection against parasites is a complex process, which is heavily influenced by unknown or uncontrollable environmental factors. The currently described host defence mechanisms are diverse and their genetic background is uncertain, as is their effective involvement in colony survival. More research is, therefore, required to improve our understanding of those host mechanisms enabling colony survival and of the influence of environmental factors on their expression. The generation interval of honey bees implies the need for selection work to span several years, which conflicts with the urgent need to identify a sustainable solution to the problem of *V. destructor*. Selection programmes focusing on survival traits in colonies infested with *V. destructor* thus need to be optimally designed to provide solutions as rapidly and efficiently as possible in terms of both time and resources. The current rate of progress suggests that strategies should be critically reconsidered so as to ensure goals are reached faster. Better programme design and, more specifically, the regular assessment of selection progress are required to optimise resource investments and accelerate progress. As failure of selection programmes is likely, due to the current state of knowledge, the sooner a failure is recognised, the faster the programme can be re-directed. Based on the review of successes and weaknesses reported to date, we have provided suggestions for such strategies (summarised in Figure 2) to jointly select survival-associated traits, evaluate genetic progress and limit losses of host genetic diversity. The better availability of information concerning the strategies, outcomes and limitations of past and current programmes should provide even better insights into how best to improve selection programmes. The more consistent publication of such information could be achieved by developing partnerships and networks between research institutes and breeders. By allowing for an increase in the number of colonies considered in selection programmes, such networks would also allow for the acquisition of more reliable data and, therefore, the development of better phenotypes enabling selection progress towards the survival of *A. mellifera* colonies infested with *V. destructor*.

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Glossary

Breeding value: corresponds to the additive genetic value of an individual, that is, the sum of the mean effects of the genes transmitted by its parents; it is used to estimate the genetic value of the offspring.

Dams and Sires: direct maternal and paternal, respectively, ascendants of an animal.

Genetic value: corresponds to the mean effect of the genotype on the phenotype, independently of the test environment.

Heritability (h^2): indicates the proportion of the phenotypic variance that is of additive genetic origin. The heritability is trait-, population-, generation- and method-specific, and it ranges between 0 and 1.

Index selection: selection according to a weighted linear combination of the relative importance of multiple traits according to the pursued breeding goal.

Natural selection: an evolutionary process, which favours the survival and reproduction of the fittest animals within the population by means of adaptation to environmental conditions, with no human intervention. Programmes based on natural selection can, however, be set up by breeders for breeding purposes.

Pedigree: list of paternal and maternal ancestors of an individual.

Phenotypic value: field-recorded value corresponding to the traits of interest being measured by the breeders.

Response to selection: progress of genetic values between generations; desired outcome of the selection program.

Selective breeding: humans intervene and pursue a breeding goal in order to identify those animals with the best performances among all the available selection candidates. These animals will be used in reproduction with the aim of increasing the performance level of the offspring population when compared to that of the parental population.

Tables

Table A: Summary of published heritability estimates for resistance traits. The study providing the estimates, the population considered and the number of colonies measured are indicated.

Trait	*Study*	*Country, population*	*Number of colonies*	*Estimate (s)*
Colony survival	No data			
SMR	Harbo and Harris, 1999a	USA	28	0.06 to 0.46 (depending on test date)
	Wielewski *et al*, 2012	Brazil, Africanized honey bees	50	0.61 to 0.63
VSH	Boecking, 2000	Germany	157	0.18 ± 0.27
Hygienic behaviour towards dead brood	Milne, 1985	Canada	69	0.144 ± 0.017 (uncapping), 0.022 ± 0.004 (removal of brood)
	Harbo and Harris, 1999a	USA	28	0.65 ± 0.61
	Boecking *et al*, 2000	Germany, *A. m. carnica*	157	0.36 ± 0.30
	Stanimirovic *et al*, 2008	Serbia, *A. m. carnica*	80	0.44 to 0.63
	Büchler *et al*, 2008	Germany, *A. m. carnica*	4281	0.29
	Ehrhardt *et al*, 2010	Germany, *A. m. carnica*	-	0.15 ± 0.03 to 0.47 ± 0.02
	Pernal *et al*, 2011	Canada	812 to 835 depending	0.17 ± 0.07 to 0.25 ± 0.10

			on repetition	
	Costa-Maia *et al*, 2011	Brazil, Africanized honey bees	40	0.10 to 0.27
	Wielewski *et al*, 2012	Brazil, Africanized honey bees	50	0.58
	Garcia *et al*, 2013	Brazil, Africanized honey bees	30	0.52
	Padilha *et al*, 2013	Brazil, Africanized honey bees	110	0.52
	Guarna *et al*, 2017	Canada	771	0.56 to 0.57
	Facchini *et al*, 2019	Italy	25	0.23 ± 0.16 to 0.37 ± 0.25
	Maucourt, 2019	Canada	ca. 900	0.18
Post-capping duration	Harbo, 1992	USA	26	0.61 ± 0.19
	Harbo and Harris, 1999a	USA	28	0.89 ± 0.59
	Jordan, 1991	Germany	-	0.6 ± 0.1
	Le Conte *et al*, 1994	France	32	0.22 ± 0.25 to 0.31 ± 0.10
	(Büchler, 1990; Büchler and Drescher, 1990)	Germany	112	0.23
	Moritz, 1985	Germany	28	0.8
Grooming	Moretto *et al*, 1993	Brazil	30	0.71 ± 0.41
	Harbo and Harris, 1999a	USA	28	0.0 to 0.17
	Stanimirovic *et al*, 2010	Serbia, *A. m. carnica*	80	0.16 to 0.49
Mite infestation level	Harbo and Harris, 1999a	USA	28	0.01 ± 0.46 (number of mites per 1000 bees) ; 0.17 ± 0.52

				(total mite population in summer)
	Padilha *et al*, 2013	Brazil, Africanized honey bees	110	0.13 (percentage of mites on adult bees)
	Wielewski *et al*, 2012	Brazil, Africanized honey bees	50	0.54 (infestation rate)
	Büchler *et al*, 2008	Germany, *A. m. carnica*	4281	0.24 (mite infestation development)
	Ehrhardt *et al*, 2010	Germany, *A. m. carnica*	-	0.32 ± 0.03 (worker effect), 0.17 ± 0.02 (queen effect)
	Boigenzahn and Willam, 1999	Austria, *A. m. carnica*	1638	0.13 ± 0.05 (mites killed by late season treatment)
	Maucourt, 2019	Canada	ca. 900	0 (natural mite fall)
Uncapping–recapping	No data			

- Data not reported in original publication.

Figures

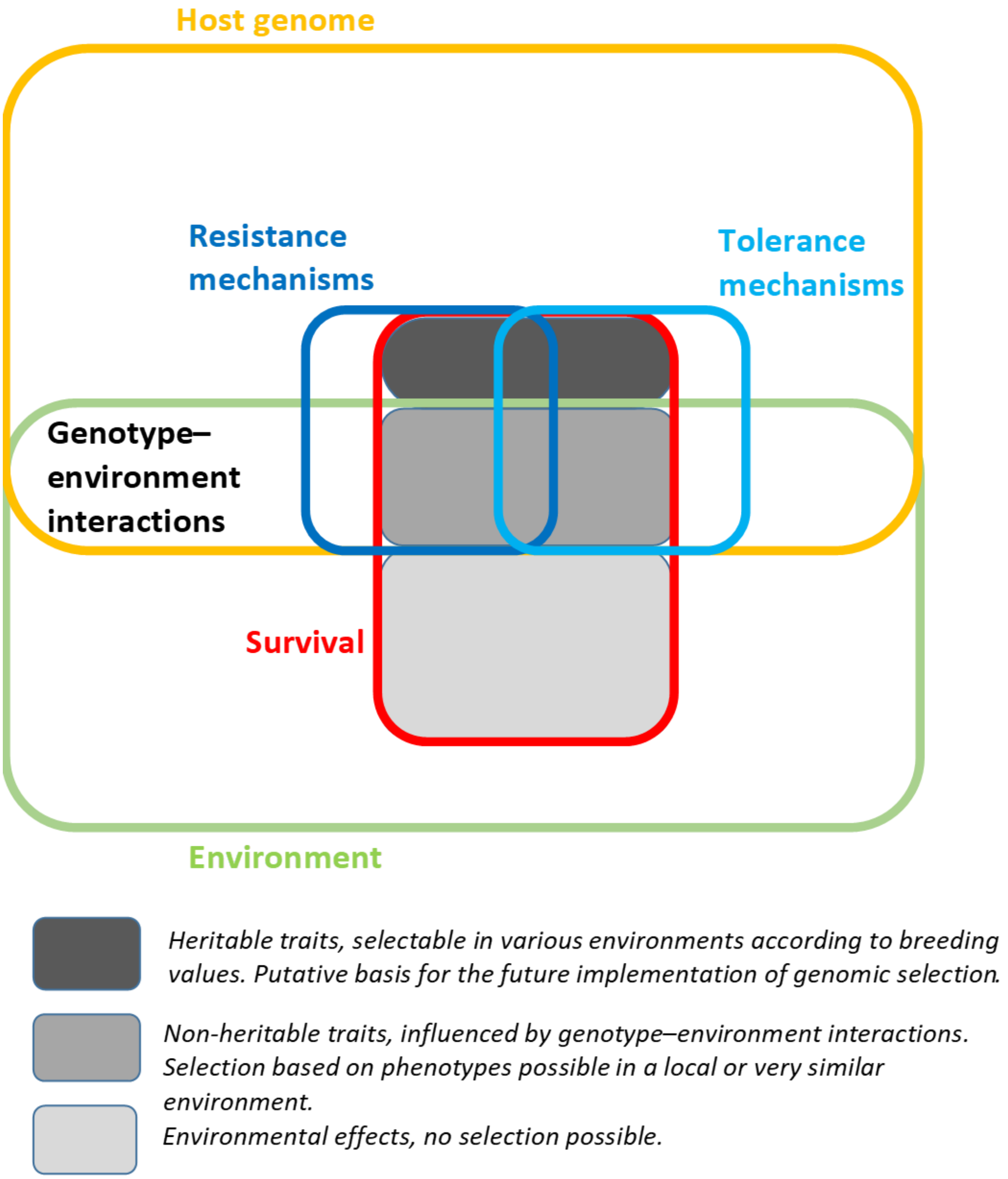
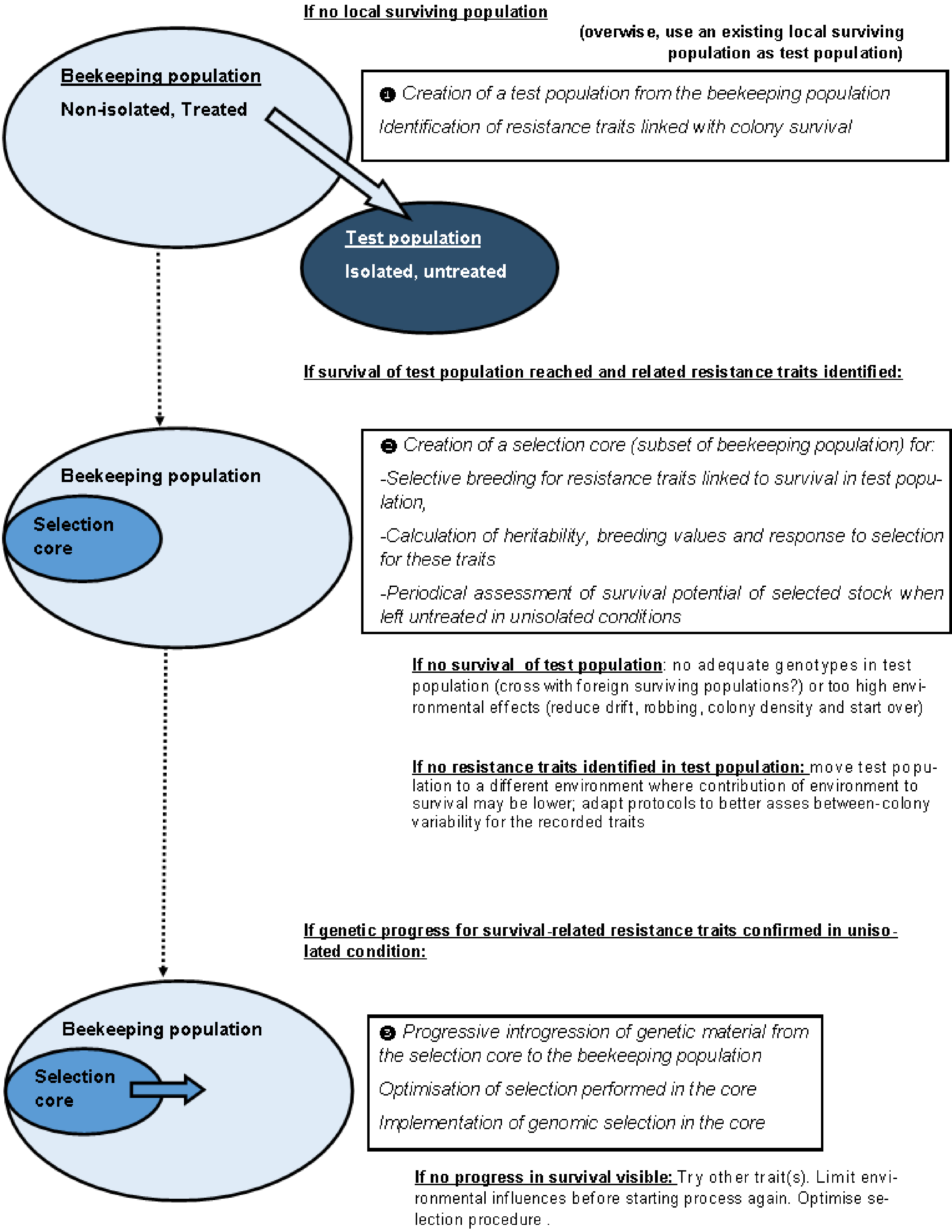


Figure 1: Theoretical model of the contributions of the *A. mellifera* genome and colony environment to tolerance, resistance and survival, as well as the implications for selection. The resistance and tolerance mechanisms (dark and light blue boxes) can both be influenced by the genome of the host (orange), and their expression level may differ depending on the genotype–environment interactions (overlapping orange/green). Some mechanisms may be linked to both resistance and tolerance (e.g. resistance to viruses may contribute to tolerance against the mite). Some resistance or tolerance traits may be expressed by the colonies, albeit

not sufficiently to enable survival in untreated conditions (areas in blue shapes but outside red shape). An important aspect of observed survival (red shape) may be of strictly environmental origin (including climate, food resources, land use, colony management by beekeeper, colony density, cavity size, virulence of the mite and its associated viruses; red shape). The dark grey area represents survival due to heritable traits (red shape), which can be selected even in variable environments. The medium grey indicates phenotypic variation, which can be selected in a given environment by including the genotype–environment interactions. The light grey indicates variation, which is only of environmental origin and so cannot be selected.



If no local surviving population

(overwise, use an existing local surviving population as test population)

❶ *Creation of a test population from the beekeeping population*

Identification of resistance traits linked with colony survival

If survival of test population reached and related resistance traits identified:

❷ *Creation of a selection core (subset of beekeeping population) for:*

-Selective breeding for resistance traits linked to survival in test population,

-Calculation of heritability, breeding values and response to selection for these traits

-Periodical assessment of survival potential of selected stock when left untreated in unisolated conditions

If no survival of test population: no adequate genotypes in test population (cross with foreign surviving populations?) or too high environmental effects (reduce drift, robbing, colony density and start over)

If no resistance traits identified in test population: move test population to a different environment where contribution of environment to survival may be lower; adapt protocols to better asses between-colony variability for the recorded traits

If genetic progress for survival-related resistance traits confirmed in unisolated condition:

❸ *Progressive introgression of genetic material from the selection core to the beekeeping population*

Optimisation of selection performed in the core

Implementation of genomic selection in the core

If no progress in survival visible: Try other trait(s). Limit environmental influences before starting process again. Optimise selection procedure .

Figure 2: Guidelines for designing programmes aiming at the selection of traits enhancing colony survival against infestation with *V. destructor* in a local honey bee population. If no local surviving population exists naturally, a test population is created from colonies reared by local beekeepers (i.e. from the beekeeping population kept under routine management, including chemical or biotechnical interventions against *V. destructor*), and its survival in untreated and isolated conditions is evaluated. Heritable traits linked to this survival are then identified and colonies from among the beekeeping population expressing such traits are selected (selection core). If a response to selection is obtained for the resistance traits and colony survival is improved, the selection core is used as a genetic resource for the introgression of resistance alleles into the beekeeping population, thereby maintaining both genetic diversity and resilience.