

REVIEW ARTICLE



***Varroa destructor*: research avenues towards sustainable control**

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Summary

Pollination by honey bees plays a key role in the functioning of ecosystems and optimisation of agricultural yields. Severe honey bee colony losses worldwide have raised concerns about the sustainability of these pollination services. In many cases, bee mortality appears to be the product of many interacting factors, but there is a growing consensus that the ectoparasitic mite *Varroa destructor* plays the role of the major predisposing liability. We argue that the fight against this mite should be a priority for future honey bee health research. We highlight the lack of efficient control methods currently available against the parasite and discuss the need for new approaches. Gaps in our knowledge of the biology and epidemiology of the mite are identified and a research road map towards sustainable control is drawn. Innovative and challenging approaches are suggested in order to stimulate research efforts and ensure that honey bees will be able to sustainably fulfil their role in the ecosystem.

***Varroa destructor*: alternativas para su control sostenible**

Resumen

La polinización por las abejas melíferas tiene una importante relevancia en el funcionamiento de los ecosistemas y en la optimización de los rendimientos agrícolas. Se ha expresado la preocupación acerca de la sostenibilidad de estos servicios de polinización, debido a las graves pérdidas de colonias de abejas melíferas a nivel mundial. En varios de estos casos, la pérdida de estas abejas parece ser el resultado de la interacción de muchos factores, existiendo un creciente consenso en que el ácaro ectoparásito *Varroa destructor* desempeña alguna

responsabilidad predisponente. Proponemos que la lucha contra éste ácaro debe ser una prioridad para futuras investigaciones relacionadas con la salud de las abejas. Resaltamos la actual necesidad de contar con métodos de control eficientes contra éste parásito y discutimos también la necesidad de nuevos enfoques. Se identificó la falta de conocimiento acerca de la biología y epidemiología del ácaro y se propuso una ruta de investigación para su control sostenible. Se sugieren desafíos con nuevos e innovadores enfoques con el fin de estimular los esfuerzos de investigación y procurar que las abejas melíferas sean capaces de cumplir de manera sostenible su relevante función en el ecosistema.

Keywords: honey bee, *Apis mellifera*, pollination, colony losses, *Varroa destructor*, sustainable control, future perspectives

Introduction

Varroa mites (*Varroa* spp.) reproduce in the cells of developing honey bees (*Apis* spp.). They feed on the haemolymph of developing and adult bees, resulting in the transmission of secondary diseases that reduce the lifespan of infested individuals (Batuev, 1979; Ball and Allen, 1988; Yang and Cox-Foster, 2007; Dainat *et al.*, 2011). The mites shifted from their natural host, the Eastern honey bee *Apis cerana*, to the Western honey bee *Apis mellifera*, about 70 years ago, after *A. mellifera* was introduced into the native range of *A. cerana* (Rosenkranz *et al.*, 2010).

Since then, commercial transportation of colonies and natural spread have resulted in a cosmopolitan distribution of *Varroa destructor*, which has had dramatic consequences for both managed and wild populations of *A. mellifera*. *Varroa jacobsoni* has relatively minor effects on colonies of its natural host *A. cerana*, at least in part because the mite can only reproduce when male brood is present. In contrast, *V. destructor* can reproduce on both male and female brood of *A. mellifera*, thus attaining a longer reproductive season and larger mite populations. With larger numbers of mites in a colony, a greater proportion of bees and larvae are affected. Without treatment, a colony of *A. mellifera* infested with *V. destructor* dies within one to three years (Korpela *et al.*, 1993; Fries *et al.*, 2006), whereas *A. cerana* colonies are able to survive infestation by varroa mites without apparent damage.

Varroa destructor is considered to be the major pest of honey bees since it spread to *A. mellifera*. Recent studies have confirmed its substantial contribution to honey bee losses across the Northern hemisphere (Brodschneider *et al.*, 2010; Chauzat *et al.*, 2010; Dahle, 2010; Genersch *et al.*, 2010; Guzman-Novoa *et al.*, 2010; Topolska *et al.*, 2010; vanEngelsdorp *et al.*, 2011). No satisfactory solution for its control has, however, yet been found, and it has become clear that the development of enduring sustainable control measures will not happen until we have a better understanding of the fundamental biology of the parasite. Such solutions are necessary to ensure the future of the economically most viable pollinator species in a context of worldwide pollinator decline. The growing number of research and review articles on the biology and control of this honey bee pest shows the increasing awareness of its role in causing colony losses. In these publications, research directions towards sustainable solutions against varroa have not, however, been explicitly stated. Here we

provide such directions by reviewing and prioritising research avenues for which a consensus on their potential for success exists. We present a research concept based on short and long term strategies that is best tackled through a cooperative approach.

The need for significant progress in the fight against this parasite has grown more urgent, particularly since uncoordinated research efforts have not yet resulted in a satisfactory solution. This calls for joining forces and expertise. Previous collaborative initiatives have shown that joint efforts can bring significant progress in varroa research. The last large scale research effort in the fight against this parasite was supported from 1998 to 2003 by the European Community in the form of a Concerted Action (CA3686), which funded a working group for the co-ordination of research on integrated varroa control. The mission of this group was to develop alternative control methods to synthetic varroacides – well-known for their associated risks of parasite resistance and contamination of bee products (Rosenkranz *et al.*, 2010). Coordination was achieved through the promotion of research exchanges and pooling of resources and information. The concerted action resulted in the establishment of the so-called 'alternative varroa control methods' (Imdorf *et al.*, 2003; Rosenkranz *et al.*, 2010), as well as in knowledge dissemination to relevant stakeholders. These methods are based on biotechnical measures (the physical removal of the parasite), as well as judicious use of organic acids and essential oils.

Alternative methods are consistent with the principles of Integrated Pest Management, and are widely used throughout the world. Although they enhance chances for colony survival and ensure residue-free hive products (Imdorf *et al.*, 2003; Nanetti *et al.*, 2003), they show many limitations and provide mixed success (Delaplane *et al.*, 2005). Not least of these limitations is variability of efficacy of the organic acids and essential oils used due to ambient temperature sensitivity, the small margin between the lethal doses for the target (mites) and non-target (bees) and to increased labour inputs (Genersch *et al.*, 2010). As a consequence, the methods have not been globally adopted, and their effectiveness is dependent on the dedication and proficiency of individual beekeepers. In this sense they can be considered to have failed in slowing down the rising global colony losses due to varroa mites. An urgent need for innovative control methods is therefore obvious. The most promising options are based on biological control using pheromones, hormones, pathogens, predators or antagonists (Rosenkranz *et al.*, 2010; Meikle *et al.*,

2012). In addition, methods that do not involve the application of chemicals or other agents into the hives are of particular interest. Such methods do not involve the hurdles, expense and delay of registering new compounds or agents, the risk of developing resistance by the parasites against compounds or agents, or the accumulation of residues in hive products. The ideal solution would be the identification and breeding of bee strains tolerant to the parasite, but given our present state of knowledge we are not close to any such sustainable solutions.

In this article, we evaluate the current state of varroa control and identify promising new approaches. We emphasize that the basic knowledge of the mite's biology and genetics needed to develop efficient and sustainable control methods is still inadequate, we propose solutions to acquire this missing knowledge and, given the complexity of the task to solve the varroa problem, promote a collaborative approach.

Research directions towards a solution against the varroa mite

Understanding host specificity

Recent progress on the systematics of *Varroa* spp. has shown a high diversity of species and lineages that appear to be specific to particular *Apis* species or even to particular populations of a host species (e.g. Anderson and Trueman, 2000; Navajas *et al.*, 2009; Warrit *et al.*, 2006). The observed host-parasite associations may be due to historical biogeographic factors (Rueppell *et al.*, 2011), and/or linked to differences in the mites' abilities to reproduce on different honey bee species, lineages and castes. It is still unclear what determines the capability of a particular varroa lineage to reproduce on a given bee host or given brood type (male only or both male and worker), or how a switch to a new host species is accomplished.

So far, only one species, *V. destructor*, has successfully colonized *A. mellifera*. The successfully invading *V. destructor* belong to just two genetic lineages, known as the Korean and Japanese strains (Anderson and Trueman, 2000). The common observation that the Japan and Korea strains of *V. destructor* have been transported widely in Asia along with *A. mellifera* colonies, but have not established populations on the southern Asian *A. cerana*, implies the northeast Asian *V. destructor* cannot reproduce on other *A. cerana* populations. This suggests co-evolution between varroa populations and their natural hosts (Oldroyd 1999). The low genetic diversity within the *V. destructor* populations infesting *A. mellifera* suggests that this globally distributed population is the result of just two successful colonization events (Solignac *et al.*, 2005). This is a sobering thought when one considers the large number of varroa species and strains that are now sympatric with *A. mellifera* in Asia.

Clearly, identification of the cues triggering parasite reproduction is crucial for understanding host switching, selection of invasive mite lineages, and virulence. Understanding the mechanisms on which this specificity is based in the indigenous *A. cerana* host populations could give invaluable new insights into mite control, and without this knowledge, attempts at developing permanent or even long-term solutions may be futile.

Modelling approaches

Population development within host colonies is a central factor influencing the virulence of varroa parasites. It is driven by the parasite's reproduction, and methods to reduce parasite fertility are therefore of central importance. Other factors affecting population growth are experimentally difficult, if not impossible, to assess. Modelling offers the possibility of identifying behaviours or processes of bees or mites that potentially affect population growth and could therefore be candidates for control methods. Several population growth modelling tools for *V. destructor* have been developed in the past (e.g. Fries *et al.*, 1994; Martin, 2001). These differ in the range of included parameters, but converge in their general conclusions. They heavily focus, however, on mite population growth within a honey bee colony and mostly ignore the interactions with the hosts and with secondary diseases for which mites function as vector (for exceptions see Martin, 2001; Sumpter and Martin, 2004). Extended models need to include the temporal and spatial patterns of bee colony collapse, the possible conditions of parasite-host equilibrium, and the role of mite spread between colonies (Eggelbusch *et al.*, 2000) in order to become more realistic, accurate and predictive.

Biological control methods

Biological control methods could overcome some of the problems generated by chemical and alternative control options (residues, resistance, non-target effects, Meikle *et al.*, 2012). These methods can involve the use of antagonists, pathogens or predators of the pest. The behaviour and physiology of the pest can also be influenced with pheromones or hormones to the point where it disturbs its reproduction and population growth in the host. So far, among the pathogens and predators of varroa, only entomopathogenic fungi have the desired characteristics of a control agent (Chandler *et al.*, 2001). Despite the fact that they show specificity towards the mite, results of field tests have been mixed, with some research groups reporting a measure of success and other groups reporting no effect (Meikle *et al.*, 2012). Fungi of the genus *Beauveria* can be considered as natural enemies of the mite since they have been found naturally-occurring on varroa (Meikle *et al.*, 2006; García-Fernández *et al.*, 2008; Steenberg *et al.*, 2010). This could simplify future registration procedures. At present, little is known of either the ecology of entomopathogenic fungi in bee hives or the most effective formulation or application method.

The use of varroa attractants also received much attention (Dillier *et al.*, 2006). In this case too, our knowledge of basic mechanisms is lacking. Our understanding of the complex chemical and spatial determinants of varroa behaviour is still too fragmentary to lead to a satisfying control method (Dillier *et al.*, 2006) and to this date, no efficient product able to disrupt the orientation of the mite is available on the market. Research on the use of pheromones or of hormones of varroa that could be exploited to disrupt the orientation or the physiology of the mite is still in its infancy.

Selecting honey bees tolerant to the parasite

Detailed knowledge of host tolerance mechanisms to mite infestation is also necessary to improve breeding programmes for varroa tolerance. At present, selection of tolerant bees is performed blindly (using lineages showing naturally lower parasite infestation) or based on secondary mechanisms of tolerance such as hygienic behaviour (Büchler *et al.*, 2010; Rinderer *et al.*, 2010). Honey bee lines that have been selected for hygienic behaviour suffer from a general lack of acceptance in the beekeeping community (Carreck, 2011; Delaplane, 2011) and do not currently represent a sustainable solution. Once the main behavioural or physiological mechanisms of tolerance are identified, genetic markers could be used to identify strains for selection and therefore target the relevant genes or traits with more efficiency (Rinderer *et al.*, 2010). The recent sequencing of the genomes of *A. mellifera* and *V. destructor* (The Honey bee Genome Consortium 2006; Cornman *et al.*, 2010) will provide great support for this aim.

Further progress in the selection of tolerant honey bee strains might be hampered by an inadequacy of selection methods, in which the role of intra-colonial genetic diversity for colony-level tolerance is under emphasised. Current research points to the importance of multiple mating of the queen resulting in a mixture of paternal genotypes, in particular as this might maintain rare but specific genotypes crucial for disease resistance (Fuchs and Moritz, 1998; Tarry, 2003).

Negative synergetic interactions causing colony losses: varroa + X

Varroa destructor does not act on its own. Indeed, due to its ubiquity, potential interactions between this mite and other contributors to colony mortality are almost inevitable and appear to be universal (Ball, 1989; Cox-Foster *et al.*, 2007; vanEngelsdorp *et al.*, 2009; Potts *et al.*, 2010). These factors may include pathogens and other parasites, environmental stressors (e.g., malnutrition or agrochemicals), and lack of genetic diversity and vitality (Brodshneider and Crailsheim, 2010; Meixner *et al.*, 2010). Whilst the list of incriminating factors is not new, the evidence for interactions

among them is growing (e.g. Alaux *et al.*, 2010). Such interactions are of particular concern, because sub lethal effects can act synergistically and result in lethality. In particular, there is convincing evidence for negative synergistic interactions between *V. destructor* and viruses (Ball, 1989; Chen and Siede, 2007; Ribière *et al.*, 2008; Genersch *et al.*, 2010). Honey bee viruses naturally persist as low-level, incidental infections that only occasionally cause overt disease, rarely to the extent that colony survival is threatened. The epidemic-scale transmission by *V. destructor* can make them lethal to colonies. Effective mite control curbs this epidemic, bringing virus titers below threatening levels (Martin *et al.*, 2010). Mite control alone is therefore sufficient to eliminate the lethality of mite-transmitted virus infections (Martin *et al.*, 2010). Independent control of viruses themselves can, however, reduce the morbidity associated with varroa infestations and the overall pathogen pressure on colonies. Attempts at designing virus-specific controls are based on antiviral treatments and on genetic resistance of honey bees. Broad-spectrum antivirals developed for medical use have historically been cost-prohibitive for use on bees and have therefore never been tried, but this may change once cheaper generic versions become available. Specific antivirals against certain honey bee viruses, based on RNAi technology, have recently gone through field trials (Hunter *et al.*, 2010) and should be available soon. This technology could also be used against varroa by targeting genes essential for the survival of the mite (Campbell *et al.*, 2010). Work is currently underway to identify honey bee genes conferring resistance to virus infection and map these on the honey bee genome. Such information could be used either directly in breeding programmes or to develop new virus blocking strategies.

Anticipating new threats

The increasing scale of modern world trade obviously creates a health risk for honey bees. History has repeatedly shown that pests cannot be stopped at borders, which they eventually cross either naturally or via illegal or accidental imports (e.g. Goodwin, 2004). *V. destructor* is not the only mite pest of bees; several other mites (other varroa lineages or species, *Tropilaelaps* spp.) have the potential to invade and can also act as vectors for viruses in *A. mellifera* (e.g., *T. mercedesae* (Dainat *et al.*, 2009; Forsgren *et al.*, 2009)). Researchers should therefore make a head start on developing eradication or control methods against these new threats and evaluate those methods already in place against *V. destructor* for efficacy against potential newcomers.

Eradication as possible scenario

Previous successes in region-wide pest eradication suggest that such a feat is not out of the question with *V. destructor*. An interesting example exists in the case of the programme executed in the 1990's-

2000's to eradicate the cattle bont tick (*Amblyomma variegatum*) in the Caribbean (Bowman, 2006). The noteworthy parallel is the fact that each pest has only one or few reproductive hosts, *Apis* in the case of varroa and cattle in the case of the bont tick. This relationship with a narrow range of hosts is key to the success of an eradication programme, limiting pest refuges and narrowing in space and time the arena requiring treatment. Other necessities were surveillance to monitor the presence of the tick, efficacious miticide, training, extension, and perhaps most difficult, region-wide participation of livestock owners to perform the compulsory treatment. The parallels between the two systems suggest that, in principle, *V. destructor* could likewise be the target of coordinated, regionalized eradication. The fact that *V. destructor* has already eliminated most wild and feral honey bees in many localities (Kraus and Page, 1995) further strengthens the feasibility, given that fewer refuges exist outside managed apiaries. The obstacles are, however, immense, not the least of which is the necessity of coordinating such a programme at a continental level, since natural reinvasion from neighbouring infested regions would compromise the venture. Alternatively, finding a way to prevent such reinvasion (Koeniger *et al.*, 2011) would greatly improve the chances of success for an eradication programme. Ultimately, this success would depend on political will and beekeeper compliance, but given the present worldwide awareness of the problems facing the honey bee, there is no better time than now for such an enterprise. A limit to the immediate implementation of such a programme is the lack of efficient varroa control methods that do not rapidly generate resistance in the parasite population.

A lack of research tools hampers progress

Several important research directions have been identified (Box 1). A lack of efficient tools for achieving some of these goals has, however, been recognized. The group formed during the Concerted Action recognized the need for a standardized procedure to test the efficacy of varroacides, given the global distribution of the pest and number of teams involved in the research. Recommendations were therefore produced that have recently been incorporated into an official guideline for the development of varroacides and published by the European Medicine Agency (EMA/CVMP/EWP/459883/2008). Presently, given the diversity of approaches needed to work towards sustainable varroacides and the large number of researchers engaged in the topic, more standardization is required for an efficient and coordinated progress. A new initiative, called "the BEEBOOK", will be used for this purpose. It is based in the COLOSS network and is aimed at establishing standardized protocols for executing honey bee research (Neumann and Carreck, 2010; www.coloss.org/beebook). An important research tool lacking at present is a method for *in vitro* rearing of the mite. Such a tool is necessary for obtaining large quantities of mites for experiments at any time of the year. Rearing

mites in the field generates colony losses and imposes constraints in logistics and time and results in variations generated by spurious environmental vagaries. Standardized rearing methods would thus allow greater reproducibility in the investigation of factors influencing parasite physiology. Given the synergy between *V. destructor* and viruses, there is a need to better understand virus epidemiology. An important tool for this purpose is still lacking: without cell cultures to purify and propagate bee viruses, it is difficult to isolate specific strains to assess their virulence. Such cultures would also make it possible to characterize viral life cycles and molecular determinants of viral tropism and transmission. Previous work also showed that the availability of *in vitro* systems for studying viral infections greatly contributed to the development of antiviral drugs (Magden *et al.*, 2005).

Box 1. Proposed research directions towards a solution against the major honey bee pest: the ectoparasitic mite *V. destructor*.

Long term projects

- develop biological control against *V. destructor* (pheromones, entomopathogens, endosymbionts).
- identify the trigger mechanisms of *V. destructor* reproduction (on original and new host, including the geographic and genetic variation).
- develop *V. destructor in vitro* rearing method and reproduction tests.
- search for *V. destructor* tolerant bees and identify the tolerance mechanisms for breeding programmes and deal with the problem of narrowing genetic diversity.
- understand host-parasite co-evolution and local adaptations for *V. jacobsoni* and *V. destructor* on *Apis cerana*, study the role of and ensure the maintenance of genetic diversity.
- prepare for the putative arrival of new invasive mites (*Varroa* spp. and *Tropilaelaps* spp.).
- eradication programmes and border protection for *V. destructor*.
- investigate the impact of *V. destructor* invasion on virus presence in populations.
- understand virus transmission and virulence.

Shorter term projects

- screen for new varroacidal compounds (development and registration).
- improve formulation and application of existing varroacides
- Complete the *V. destructor* genome.
- Improve and develop models of *V. destructor* population dynamics.
- redefine *V. destructor* economic thresholds taking into account the effect of viruses.

Conclusion

Since the new avenues for research aimed at sustainable control of *V. destructor* constitute long term goals, it is also important to improve, in parallel, methods that are presently available (Box 1.). This is a continuation of the work done by the Concerted Action group. For example, it makes sense to continue focusing on oils or organic acids because these compounds are generally thought to have a low risk of engendering genetic pest resistance. The continuing problem of climate dependency of the alternative control methods could also be solved by the development of new formulations and/or applications of existing products.

Although *V. destructor* is not the sole cause of colony losses experienced worldwide in recent years, a consensus emerges that it represents the key factor (Neumann and Carreck, 2010). Removing *V. destructor* from the complex equation of honey bee health would reduce the pressure on the honey bee's extensive natural defence mechanisms (Evans and Spivak, 2010) against the many environmental health challenges. Using sustainable methods to control or even eradicate this parasite will re-establish wild and feral pollinator populations, ease the plight of beekeepers, promote economically important pollination-dependant agriculture and benefit natural ecosystems. For this ideal to be realized, however, a strong and sustained research effort is needed to produce the understanding necessary for an efficient and sustainable control strategy against this most important of honey bee parasites.

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