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Disentangling multiple interactions in the hive ecosystem

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The widespread losses of honeybee colonies recorded over the past number of years in the northern hemisphere represent a major concern for the beekeeping industry and, more importantly, may have a severe impact on ecological services and biodiversity. There is now a general consensus about the multifactorial origin of colony losses, but the mechanistic basis of this complex phenomenon still remains largely elusive. In this review, we propose a functional framework for interpreting how different stress agents can interact to adversely affect bee immunity and health. This provides a new background rationale in which to develop an integrated approach to bee protection, as part of a more comprehensive strategy for the conservation of insect pollinators.

An emerging 'old' problem

Reports of bee diseases caused by parasites and pathogens can be found in Aristotle's '*The History of Animals*' as well as in ancient writings by Pliny and Virgil. However, only the intensive growth of modern beekeeping has brought about the first reports of extensive honeybee colony losses [1]. In many cases, the causes of such losses remained obscure, so that generic names referring to the location where the problem was observed, or to the season of its occurrence (e.g., Isle of Wight disease or May disease [1]) were often used to define them. These poorly defined syndromes were repeatedly observed over the years; however, the biggest sanitary emergency that the beekeeping industry has faced so far came to the fore only at the beginning of this century.

In 2006, the first records of mysterious die offs of honeybee colonies were reported in the USA [2], soon followed by similar reports of colony losses from all over the northern hemisphere [3]. Systematic monitoring activities in Europe and the USA have shown that losses in the range of 20–30% of colonies are rather common, even though much higher rates can occur locally [4,5]. In the USA, losses were initially attributed to a complex syndrome, denoted as colony collapse disorder (CCD) [6], which is characterized by symptoms not observed elsewhere [3]. Indeed, CCD is still unreported in Europe [7], whereas it is now regarded as one of the many causes of colony losses in the USA [5].

Reported losses could reinforce the decline in number of managed bee colonies recorded over the past few decades in these geographic areas [8], which, in the long term, may have dramatic consequences for the environment and agriculture, given the pivotal contribution of animal pollinators to plant biodiversity and crop production [9]. The concurrent decline of wild pollinators [10,11] that play a role that is not yet fully recognized [12] adds up even more reasons for concern.

From one possible causal agent to many

The identification of the possible causal agents responsible for bee colony losses has attracted increasing interest in the scientific community. Most of the early studies attempted to correlate the state of collapsing colonies, sampled under a vast range of environmental conditions, with the presence of potential risk factors (see, e.g., [13]). In this way, different stress factors, such as viruses, pesticides, parasites, and fungal pathogens, were, in turn, identified as the main causal agents for colony losses [14]. However, subsequent studies often revealed that, while involved, these factors were likely not responsible on their own for the observed losses, but were part of a complex multifactorial syndrome [14]. This hypothesis postulates that the decline of the honeybee colony and its eventual collapse can be induced by a variety of stress factors, often showing synergistic interactions (Table 1). However, the mechanistic basis of this complex phenomenon still remains largely elusive.

Unraveling the intricate interactions between different parasites and stress factors, which underpin the multifaceted dysfunctions of challenged organisms, is extremely difficult [15]. However, in the case of honeybees, the intricate network of interactions between different stressors is very often characterized by the occurrence of Deformed Wing Virus (DWV), which can generate covert infections readily spread by the parasitic mite *Varroa destructor* [16,17], as predicted by the model proposed by Martin [18]. This epidemiological scenario has been corroborated by a study carried out in the Hawaiian islands, which showed that the arrival of the mite resulted in a dramatic increase over time of DWV prevalence and loads, and in the selection of an aggressive viral strain [19]. More recently, Ryabov *et al*. [20] showed that only a virulent recombinant

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Table 1. Major factors affecting honey bee health and possibly involved in honey bee colony losses

Factor	Details	Impact on honeybees	Essential Refs
Deformed Wing Virus (DWV; Picornavirales, Iflaviridae)	Single stranded RNA virus	High titers of DWV cause deformities and reduce bee survival	[16]
Varroa destructor (Acari, Varroidae)	Bloodsucking parasite	The mite feeds upon the bee hemolymph, causing direct damage and vectoring/ activating bee viruses	[17]
Nosema ceranae (Microsporidia, Nosematidae)	Spore-forming unicellular parasite	Infects the ventriculus of adult honey bees	[59]
Additional bee viruses (Picornavirales, Iflaviridae, and Dicistroviridae)	Single stranded RNA viruses	Cause variable symptoms	[60]
Agrochemicals	Several pesticides including neonicotinoids and acaricides used for mite control	Induce both lethal and sublethal effects	[61,62]
Landscape fragmentation and deterioration	Loss of spontaneous vegetation and diffusion of crop monoculture	Affect adequate and continuous supply of food sources	[63]

form of DWV actively replicates when introduced in the honeybee hemocoel, either by *Varroa* feeding or artificial injection; this results in a dramatic reduction of DWV diversity in honeybees, in contrast with the high level of DWV diversity observed in mites, where active viral replication concurrently takes place. Therefore, it seems that the route of virus acquisition is essential to trigger the intense amplification of a virulent strain of DWV in *Varroa* infested colonies, even though the molecular mechanism conferring the advantage to this near-clonal DWV strain remains obscure.

Whatever the causes promoting DWV replication, the resulting high viral loads determine honeybee reduced survival and the appearance of developmental deformities, which are reliable markers of overwintering colony losses [21]. This clearly indicates the central role played by DWV which, by spreading asymptomatically, becomes the 'Achilles heel' of any infected colony, constantly exposed to the risk of explosive viral proliferation in response to stress.

A model of interactions

The study of the molecular changes occurring in bees exposed to the combined action of the *Varroa*–DWV association has been the focus of numerous research efforts. The emerging common theme is the occurrence of a dysfunctional honeybee immune system, which shows conserved insect traits, as well as specific peculiarities associated with eusociality (Box 1). However, the key role proposed for *Varroa* in the induction of this immune syndrome [22] has been questioned [20,23–26]. New evidence has indicated that DWV exerts an immunosuppressive action [27] at very high levels of viral load (exceeding 10^{15} DWV genome copies), characterized by a transcriptional downregulation of several immune genes, including a nuclear factor-kappaB (NF-κB) transcription factor, which plays a central role in immunity and regulates defense pathways activated by different stress agents [28,29]. Even though the antiviral barriers in insects are largely dependent on RNAi mechanisms [30,31] that appear to remain active in CCD colonies [32], Janus kinase–Signal Transducer and Activator of Transcription (JAK–STAT) signaling pathways and NF-κB dependent immune pathways have also been described [33–37], and are considered to be possible targets of honeybee viral pathogens [32]. In fact, the experimental silencing in honeybees of *dorsal-1A*, a gene encoding a member of the NF-κB protein family, triggers DWV replication [27], and the antiviral response by nonspecific double-stranded RNA (dsRNA) is associated with significant downregulation of immune factors under NF-κB transcriptional control [38]. Moreover, honeybee pupae exposed to *Varroa* and with high DWV loads (not exceeding 10^{10} genome copies) also show the transcriptional downregulation of immune genes in the Toll pathway, even though the overall response is complex and the role of modulated genes remains to be further explored [20].

Overall, the available data clearly indicate that the immune system of honeybees exposed to the combined action of the *Varroa*–DWV association is somehow compromised, but the variable response observed at different infection levels suggests that the dynamic nature of the host–virus interaction may have a significant impact on the molecular interplay between DWV and infected honeybees. Indeed, the complex dynamical properties of the DWV–honeybee interaction have been largely overlooked. The model that best describes the observed bi-stable DWV dynamics (i.e., low DWV copy number associated with covert infections, and high copy number causing overt infections) requires that the immunosuppressive effect of DWV displays some form of threshold function with increasing viral copy number, which can promote a sudden transition from a covert infection to explosive viral growth [27]. This allows the virus to function as an opportunistic pathogen, able to sense increasing host stress/weakness, before adopting an aggressive exploitation strategy, characterized by escalating immunosuppression and explosive growth. This kind of bi-stable dynamics, preventing uncontrolled replication when the viral load is below a critical threshold, allows asymptomatic virus persistence and favors its spread in the honeybee populations, either by