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IN AND OUT OF THE BOX: RESISTANCE TO *VARROA* AND REWILDING IN THE NETHERLANDS

Dentro e fuori l'arnia: resistenza alla Varroa *e reinselvatichimento in Olanda*

A parasite, from the Greek 'parasitos': one who eats at the table of another, is a species that depends of another species for its food, shelter and/or reproduction, having a cost for its host in the process. Most living organisms are parasites and it is estimated that much of the genetic diversity we see on the planet is shaped by host-parasite coevolution (WINDSOR, 1988).

Varroa destructor is a mite species originally restricted to *Apis cerana*, the Asian honeybee with which it coevolved. In the mid-twentieth century, *Varroa* successfully switched host as the Western honeybee *Apis mellifera* was introduced in Eastern Asia for beekeeping. Restricted to the drone brood in *Apis cerana*, *Varroa* mites can reproduce in both the worker and drone brood of *Apis mellifera* resulting in high infestations that can kill colonies in typically 2 to 3 years (ROSENKRANZ *et al.,* 2010).

As *Varroa* spread to Europe in the 1970's and 80's, the important colony losses following were, understandably, met with systematic control of the parasite through chemical or mechanical means. Although efficient to some extent, the control of *Varroa* has the disadvantage of reducing the parasite pressure on honeybee populations, thus never constraining them to adapt while on the other hand *Varroa* keeps adapting to chemical treatments, constantly reducing the options left to beekeepers (NEUMANN & BLACQUIERE, 2017).

Varroa destructor and *Apis mellifera*: a rapid observed coadaptation

In 1999, a pioneer experiment led by FRIES *et al.* (2006) in which 150 honeybee colonies infested with *Varroa* were left to coevolve freely in the Swedish island of Gotland demonstrated that, although with high initial colony mortality rates, *Apis mellifera* could survive in the presence of *Varroa*.

Inspired by these results, the researchers and beekeepers Tjeerd Blacquière, Willem Boot and Johan Calis started developing and using in 2007/08 a protocol based on natural selection to keep honeybees in the absence of *Varroa* control while avoiding important losses for the beekeepers (BLACQUIÈRE *et al.,* 2019). Tjeerd Blacquière will explain the principles of this 'Darwin Black Box' protocol during his talk: selection is done solely on survival, growth and reproduction and the content of the black box -the mechanisms contributing to survival- is not important for the selection and could remain unknown. Scientists have, however, regularly opened the box to investigate traits associated with resistance to Varroa.

Inside the black box: how do honeybees survive *Varroa* infestations?

After several years of selection in the absence of Varroa control, a stable number of colonies were able to survive by maintaining a decreased mite population growth. Thanks to the work of many colleagues, students and collaborators, some light was shed on the mechanisms contributing to this resistance.

Traits linked to brood signals such as suppressed mite reproduction (SMR) (BROECKX *et al.,* 2019) as well as behavioural mechanisms such as *Varroa* sensitive hygiene (VSH) (PANZIERA *et al.,* 2017) appear to play a role in the resistance of these colonies to *Varroa*. While grooming and recapping are reported in other populations, they seem to be absent in the Dutch resistant populations (KRUITWAGEN *et al.,* 2017; MORO *et al.*, 2021a). Changes in the genetic structure of *Varroa* mite populations associated with resistant honeybee colonies show that coevolutionary dynamics between host and parasite might be critical (MORO *et al.*, 2021b). Exposure to *Varroa* also implies exposure to its vectored viruses, resulting in an adapted tolerance (LOCKE *et al.,* 2021). Overall, *Varroa* resistance seems to rely on a diversity of traits, that may themselves vary overtime (MORO *et al.*, 2021b).

Bed&Breakfast: rewilding honeybees in intensively managed landscapes

In Africa, honeybee populations are composed of both colonies managed by beekeepers and wild colonies, with very seldom artificial breeding practices. Concurrently, African honeybee populations appear largely resistant to the major diseases (DIETEMANN *et al.,* 2009), highlighting the importance of wild colonies to maintain sustainable and healthy populations of honeybees (MORITZ *et al.,* 2007).

Apis mellifera is autochthonous to Europe but it is commonly assumed that the majority of wild-living colonies has been eradicated with the introduction of *Varroa* in the 1980s and 1990s. Nevertheless, several recent publications documented cases of wild-living colonies in German beech forests (KOHL & RUTSCHMANN, 2018) and in urban areas of Eastern Europe (OLEKSA *et al.*, 2013; BILA DUBAIC *et al.,* 2021).

These encouraging reports prompted us to attempt the reintroduction of wild-living colonies in the Netherlands, where the landscape is heavily modified by human activity. We hypothesised that parasite susceptibility and the lack of large trees in which to nest are the main limits to the establishment of a feral honeybee population. We thus started a rewilding project using *Varroa*-resistant colonies allowed to swarm freely within protected natural areas. Nesting boxes are placed in trees with the hope that these 'beds', together with a good 'breakfast' forage will stimulate the establishment of swarms and their survival.

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