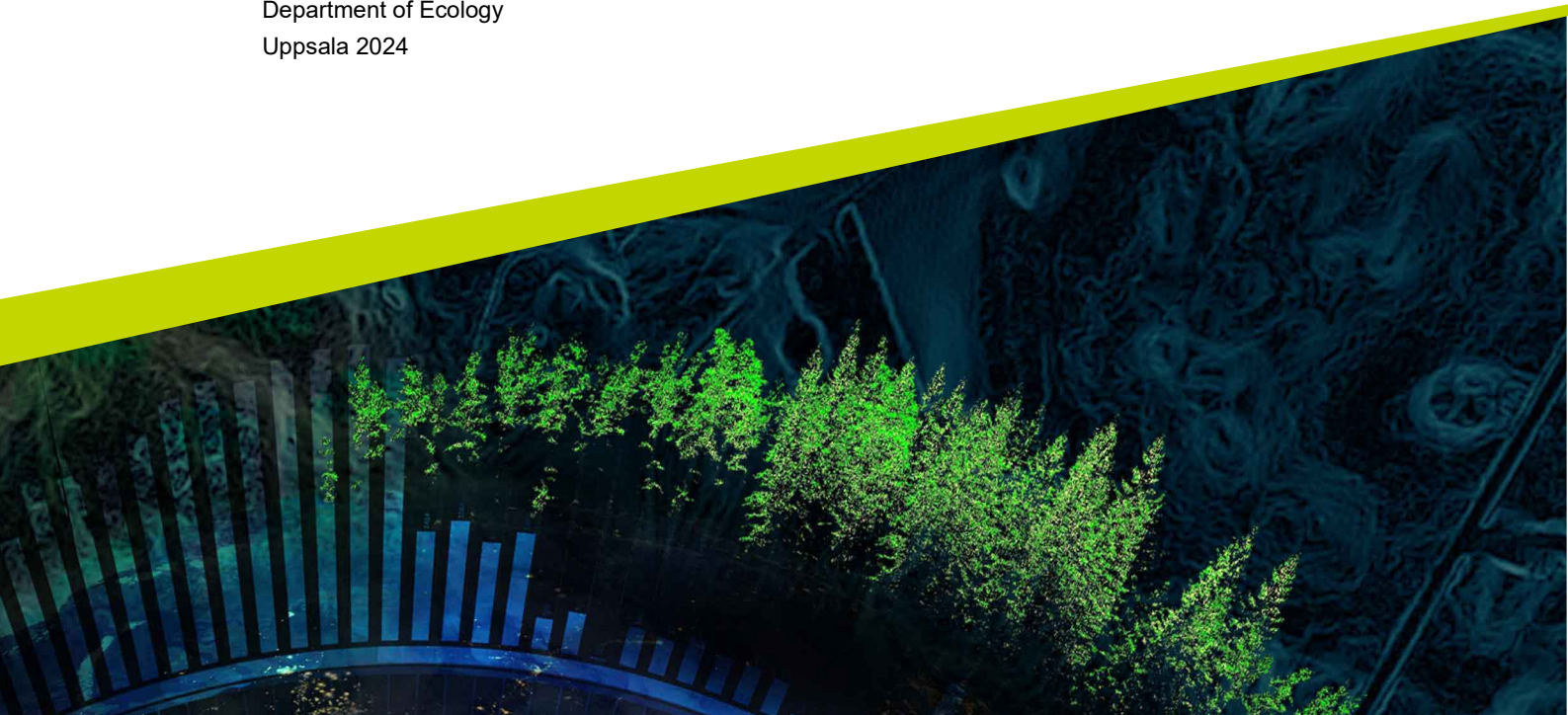




The Varroa invasion and the resistance fighters

Edgar Stigell

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Swedish University of Agricultural Sciences, SLU
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Department of Ecology
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Varroainvasionen och motståndsrörelsen

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Abstract

The European honeybee, *Apis mellifera*, is essential for global agriculture, significantly contributing to the pollination of 35% of agricultural land and supporting the production of 87 leading food crops. However, the species is under threat from the ectoparasitic mite *Varroa destructor*, which originally parasitized the Asian honeybee, *Apis cerana*, before making a host switch approximately 65-105 years ago. This switch has led to the rapid and widespread infestation of *A. mellifera* populations, causing severe colony losses worldwide, with USA losses reported at 40.5% in 2015-2016, rising to 45.5% by 2020-2021.

Varroa impacts its host by feeding on haemolymph and body fat, reducing bee weight and lifespan, and by transmitting viruses such as Deformed Wing Virus (DWV), which causes high brood mortality and deformities. Two different host switches have resulted in two primary haplotypes: the less virulent Japanese and the more destructive Korean, with the latter spreading throughout Europe, Asia, and the Americas.

Given the limited natural defences of *A. mellifera* against *Varroa*, current control methods involve the use of various acaricides. However, these methods face challenges such as resistance development and potential contamination of honey and other bee products. This report aims to synthesize existing knowledge on naturally resistant *A. mellifera* populations and their traits, providing an overview of these traits and the status of feral colonies that survive without treatment. By examining these resistant populations and their adaptive behaviours, this report seeks to inform and enhance sustainable management strategies against *Varroa* in global apiculture.

Keywords: Honeybee, *Varroa*, Natural Resistance, Parasite

Table of contents

List of figures	6
1. Introduction	7
1.1 General background of <i>Apis Mellifera</i> importance.....	7
1.2 Varroa Destructor's effect on the global apiculture.....	7
1.3 Varroa's lifecycle	8
1.4 Varroa's effect on individual host bees	8
1.5 Host switch and the beginning of the epidemic	10
1.6 Usual methods of controlling Varroa mites in <i>A. mellifera</i> apiaries	11
1.7 Naturally resistant <i>A. mellifera</i> populations.....	12
1.8 Resistance traits in <i>A. mellifera</i>	13
1.8.1 Hygienic	13
1.8.2 Grooming	13
1.9 Aim of this work.....	13
2. Results	15
2.1 Studied Naturally Surviving <i>A. mellifera</i> populations	15
2.1.1 The African honeybee in South America and South Africa	15
2.1.2 Island of Fernando de Noronha, Brazil.....	16
2.1.3 Primorsky, Russia.....	17
2.1.4 Avignon, France.....	17
2.1.5 Arnot Forest, USA.....	18
2.1.6 Østlandet, Norway	19
2.1.7 United Kingdom	20
2.1.8 Cuba	20
2.1.9 North County Dublin, Ireland	21
2.1.10 Gotland, Sweden	22
2.2 Feral Honeybee Populations.....	23
2.3 Citizen Science: The future of Varroa research.....	24
3. Discussion	25
3.1 Behavioural traits	25
3.2 Conclusion	27
References	28

List of figures

Figure 1. A grid of the most common resistance traits documented in these Varroa-resistant populations. Green and YES if the population exhibits the resistance trait, red and NO if no difference has been found from susceptible population and blank if the trait has not yet been investigated.....27

1. Introduction

1.1 General background of *Apis Mellifera* importance

Pollination is a vital part in the reproduction of the world's many flowering plants, allowing sexual reproduction, and increasing genetic variation (Kevan & Viana, 2003).

In an article released in 2011, Ollerton et al. estimate that of the ca 352 000 species of flowering plants that interact with pollinators, over 300 000 of them are pollinated by animals. The pollination performed by these animals is a vital ecosystem service, providing both direct and indirect benefits for humanity (Fisher et al. 2009). Out of all the pollination performed by animals, around 99% of it is made by insects (Hoshiwa & Masami, 2008). Insect pollination is also essential to agronomy, with pollinators affecting 35% of global agricultural land, supporting the production of 87 of the leading food crops worldwide (FAO, 2018). 75% of globally imported crop species benefit in some way from insect pollination, with honeybees being one of the most prevalent species (Klein et al. 2007).

European honeybees, *Apis mellifera* is responsible for a substantial increase in food throughout the USA, with the total yield increase estimated to be worth \$14.6 billion during the year 2000, with the trend showing a yearly increase of 3.3% (Morse & Calderone, 2000).

1.2 Varroa Destructor's effect on the global apiculture

Recently the European honeybee have been subject to infestation of a new ectoparasitic mite called *Varroa destructor* hereby after referenced as Varroa unless specified otherwise. The mites natural host is the Asian honeybee *Apis cerana* and made the host switch to the naive *A. mellifera* around 65-105 years ago (Oldroyd, 1999). Since then, it has successfully invaded almost the entire global bee population, with a recent reported outbreak in Australia (NSW DPI, 2024), only some African countries and a few islands are yet to report Varroa infestation as of 2020 (Noel et al. 2020).

The devastating effects of the Varroa invasion can be seen across the globe. In the USA, Varroa is reportedly the leading driver of colony mortality, causing collapse of highly infested colonies if left without treatment (Kulhanek et al. 2017, Steinhauer et al. 2021). The average loss of honeybee colonies in the USA from 2015-2016 reportedly was 40.5% (Kulhanek et al. 2017). The mortality

increased to 45.5% five years later from 2020-2021 (Steinhauer et al. 2021), with beekeepers surveyed both years holding Varroa as the top culprit. Similar high mortality trend can be found across the globe with one survey from Uruguay showing an average winter colony loss of 18.3% in the years 2013-2014, with 61.5% of the losses being caused by parasites and diseases (Antunez et al. 2017).

1.3 Varroa's lifecycle

The Varroa lifecycle starts when a mother or “foundress mite” chemically identifies an uncapped brood cell to infest just a few hours before it is capped (Nazzi & Le Conte, 2016). The foundress mite then climbs into the jelly at the bottom of the cell, potentially to hide from the nurse bees that regularly inspect brood cells before they are initially capped (Traynor et al. 2020). After capping, when the bee brood have finished its food, stretched out in the cell, and made its cocoon, the mite climbs onto the brood and makes a hole in its cuticle to feed both herself and her future offspring. The mother mite first lays a male egg followed by 1-3 female eggs which all feed from the brood. A few days after hatching the male will then mate with all the females, who will store the sperm, enabling them to lay their own eggs later (Rosenkranz et al. 2010). When the bee brood hatches from the cell the mother mite along with usually 1-2 daughter mites are carried with it, ready to infiltrate new cells (Rosenkranz et al. 2010).

The initial hypothesis was that the mites fed exclusively on bee haemolymph, but this was later questioned based on research of the mite's mouthparts and digestive tract that suggested their diet included semisolid tissue (Griffiths. 1988). This hypothesis was further supported with findings that Varroa egg production requires the parasite to feed from the bee bodyfat (Ramsay et al. 2019) and that body-fat tissue samples were consistently found in digestive tracts of mites parasitising adult bees (Ramsey et al. 2018). A recent paper conducting metabolomic profiling of Varroa in reproductive contra dispersal stages found that reproductive foundress mites primarily consume the haemolymph of the bee pupae while Varroa in their dispersal stage primarily feed of abdominal membranes to access body fat of the adult bees (Han et al. 2024).

1.4 Varroa's effect on individual host bees

Varroa is an obligate parasite with no free-living phases during their lifecycle. Sexual dimorphism within Varroa is noteworthy, with the males being distinguishably smaller and having longer legs in relation to their body size (Rosenkranz et al. 2010). The lifecycle also differs between male and female mites. The female mites have two distinct phases throughout their life, a

reproductive phase in the sealed brood cells and a dispersal phase attached to adult bees (Rosenkranz et al. 2010). The Varroa males are short lived and can only be found in brood cells. While the mites can reproduce in both honeybee drone and worker brood cells, they show a strong preference to drone brood cells which have a longer post-capping period which results in more time for the foundress mite to lay eggs and a higher reproductive success for the mites (Fuchs. 1992).

The presence of feeding foundress mite and her brood results in bee pupae with significantly reduced haemolymph during important developmental stages for the bee. A single infesting mother mite causes an average of 7% lower bodyweight in the hatching worker bee, but the possibility of several foundress mites always exists, which would cause even greater losses (De Jong et al. 1982). One study revealed that 6% of infected workers showed physical deformation in the form of wing damage and that the bees had a significantly reduced lifespan and started foraging earlier (De Jong et al. 1982). The affected foraging worker bees also showed a lower rate of return to the colony, generally longer absences from the colony, and a decreased mental capacity regarding non-associated learning (Kralj & Fuchs. 2006, Kralj. 2007). The effect is even higher in infected drone brood where 11-19% bodyweight reduction have been documented (Duay et al. 2003) resulting in worsened flight capacity for the grown drones (Duay et al. 2002).

The effects of Varroa infestation does not stop there however, arguably the biggest impact and greatest danger for the colony comes from the mite's capacity to act as a vector for several honeybee viruses. Before Varroa's host switch and introduction of the vector, viruses were not thought as much of a danger to European honeybees (Rosenkranz et al. 2010). Varroa has been reported to carry a row of different honeybee virus, including Kashmir bee virus, Acute bee paralysis virus, Israeli acute paralysis virus, Sacbrood virus, and most prevalent, Deformed Wing Virus (Boecking and Genersch, 2008). Both the number of afflicted bees and the severity of the symptoms caused by the viruses have increased along with Varroa infestation rates (Rosenkranz et al. 2010).

Deformed Wing Virus (DWV) is the most impactful virus vectored by the mites. Without being vectored by Varroa the virus does not have a clear negative effect on the host's fitness and causes no visible deformities (De Miranda & Genersch, 2010). The mortality of honeybee brood infected with DWV from Varroa while still in its cell is very high (De Miranda & Genersch, 2010). The bees that survive often emerge from their cells with symptoms like discoloration, bloated and shortened abdomen and the cause of the virus name, deformed wings (De Miranda & Genersch, 2010). The afflicted honeybee often has a short lifespan, and its deformed wings compromise its ability to fly (Kielmanowicz, 2015). The rate of deformed bees in a hive gives some indication to how large the infestation has progressed, with the detection of 11 afflicted bees in a colony of

15 000 during autumn indicating a low chance of the colony surviving winter hibernation (Dienat & Neumann, 2013).

1.5 Host switch and the beginning of the epidemic

A. mellifera does not have any natural parasitic brood mites but have been found to be susceptible to both *V. destructor* and its close relative *Varroa jacobsoni* (Roberts et al. 2015). The two mite species both naturally parasitise the Asian honeybee and were initially believed to be the same species. This was largely due to them sharing many morphological traits that distinction between them required genetic testing (Anderson & Trueman, 2000).

The host switch to *A. mellifera* seems to have happened on at least two separate occasions (de Guzman et al. 1997, Olroyd. 1999). *Varroa* was reported parasitising western honeybees in Japan by 1957 where western honeybees already had existed since 1887 (Sakai & Okada 1973) and *Varroa* had been documented in 1909 (Suzuki. 1909). *Varroa* then spread from Japan to Paraguay in 1971, then Brazil in 1972 and dispersed to North America from there (de Jong et al. 1982). The second known host switch occurred in what was then the far east of the Soviet Union in an area called Vladivostok where *A. mellifera* colonies were introduced from Ukraine to increase honey production. There the *A. mellifera* communities were close enough to infected Asian honeybees to enable a host shift and infected western honeybees were later identified in the European part of the Soviet Union in 1975 (Crane. 1978). From there the parasite spread throughout the rest of Europe and later the world.

The *Varroa* infesting *A. mellifera* today consists of two known haplotypes, the Japanese and the Korean haplotypes, named after the country the type was first identified parasitising *A. cerana* (Anderson & Trueman, 2000). The Japanese haplotype, that originated from the host switch that was first observed in Japan is much less virulent and is contained to Japan, Thailand and parts of the Americas (Traynor et al. 2020). The Korean haplotype is thought to originate from the host switch that took place in the far eastern Soviet Union and is much more virulent than the Japanese haplotype (Solignac et al. 2005). With the Korean haplotype having spread throughout Europe, Asia and most of the Americas, there are few places with only the Japanese haplotype (Solignac et al. 2005).

One of the reasons that *Varroa* has such a devastating effect on *A. mellifera* is that it is a naive host and have not been under any evolutionary pressure to develop resistance to *Varroa* before the host switch occurred. The mite's natural host *A. cerana* does not suffer the same devastating effect from *Varroa* infestations. This is because the Asian honeybee has coevolved with *Varroa* and has had the time to adopt several defence adaptations increasing its resistance to the parasite (Rath, 1999). These defensive traits include reducing the mite's

reproductive success through increased hygienic behaviour, mite infertility in worker brood and entombment of brood infested with several mites (Guichard et al. 2020).

1.6 Usual methods of controlling *Varroa* mites in *A. mellifera* apiaries

To prevent loss of colonies many human managed apiaries use treatments to limit *Varroa* infestation. Several acaricides exist to kill the mites with varying effectiveness and intensities, from natural and essential oils and organic acids to synthetically produced chemical pesticides, all of these are collectively called varroacides. The acaricide treatment usually occurs periodically depending on mite population growth and risk of reinfestation from other beehives, with treatment not being done during nectar flow or winter and special care is taken in preparation of winter to make sure the colony survives the harsh conditions (Rosenkranz et al. 2010). In Spain as well as several other countries it has been mandatory to at least once a year treat apiaries with an acaricide to limit the spread of the mite (Hernandez et al. 2021).

Natural organic acids are often used within the EU where restrictions on pesticides are stronger than other countries. Oxalic acid and formic acid are the two organic acids foremost used as varroacides with lactic acid sometimes finding use in smaller apiaries (Rademacher, 2006). Oxalic acid has several different methods of application. The three most popular being trickling, where a syringe filled with oxalic acid dihydrate solution is trickled directly on the bees between combs, evaporation, where the acid is evaporated to fill the hives and lastly spraying, where an acid solution is sprayed onto the bees on both sides of the combs as well as the bees resting on the walls (Rademacher, 2006). While formic acid also has several different application methods the most dominant is evaporating 60-85% formic acid in the hive (van der Steen & Vejsnæs, 2021). The natural oil thymol is also used to control *Varroa*, often in combination with oxalic or formic acid (Calderone, 1999). These natural compounds come with several advantages, such as sufficient efficiency, being able to kill the mites without being too potent and having critical effects on the bees, with formic acid being the only treatment able to kill mites in sealed brood cells (Fries, 1991). They also show low risk of contaminating bee products including honey which some of them are naturally contain (Bogdanov, 2006), and lastly having a low risk of producing resistant mites even after repeated treatment (Bogdanov, 2006). However, they do have limitations, such as some of them, including oxalic acid have to be applied when the hive does not have brood (Higes et al. 1999), and the efficiency of some compounds vary greatly depending on the conditions both outside and within the hive, forcing the beekeepers to consider many factors

before choosing compounds and method of application for optimal effect (Rosenkranz et al. 2010).

Synthetic acaricides recently used to combat *Varroa* include the organophosphate coumaphos, the pyrethroids tau-flavinate and flumethrin and the formaminade amitraz (Rosenkranz et al. 2010). These pesticides have been labelled as “Hard acaricides” indicating their effectiveness and severity of the treatment. The use of these treatments has several upsides, including being easy to apply, not requiring a large amount of knowledge about the mites biology, being economically available, and being very effective varroacides (Rosenkranz et al. 2010). These stronger treatments also come with several disadvantages however, they can persist in wax and harm bees exposed to several of them (Chauzat et al., 2009, Johnson et al. 2009) as well as pollute honey and other bee products, in some cases to the degree that exceeded the EU Maximum Limit of Residue (Wallner., 1999, Martel et al. 2007). Another danger from using these acaricides comes from the development of resistance in the mites, with evidence of resistance towards fluvalinate being reported already in 1994 (Milani, 1994), with other accounts of resistance towards coumaphos and amitraz being reported (Elzen et al., 1999, Trouiller, 1998). The several downsides like pollution and specificity of varroacides along with the looming threat of the mites developing resistance all highlight the need for alternative means to protect honeybees from *Varroa*. One possible solution is presented by the mite’s original host, where mite infestation rarely becomes so devastating as to cause colony collapse because of the honeybees naturally evolved resistance to the parasite.

1.7 Naturally resistant *A. mellifera* populations

Several naturally resistant bee populations have been identified and studied worldwide. These resistant populations have been compiled and reviewed previously by Locke, 2016 and Le Conte et al. 2020. But new resistant populations have been documented (Hawkins & Martin., 2021, Luis, 2022) and the situation of feral colonies have gotten much attention lately (Moro et al. 2021, Lorenz & Rutschmann, 2018., Rutschmann et al. 2022) possibly presenting completely new opportunities to the field. This highlights the need for a new synthesis of the known documented *Varroa*-resistant population and the traits that allow their resistance.

1.8 Resistance traits in *A. mellifera*

1.8.1 Hygienic

A common behavioural defence among bees is hygienic behaviour. This is where dead, diseased, and parasitised brood are uncapped and expelled from the hive (Ibrahim & Spivak. 2006). A specific form of this behaviour, one of the resistances that the Asian honeybee has is VSH (Varroa Sensitive Hygienic) behaviour, similar to normal hygienic behaviour except varroa infested brood are more effectively identified and uncapped early to be removed from the hive (Ibrahim & Spivak. 2006; Harris. 2007).

This behaviour is exhibited in varying frequencies in *A. mellifera* bees as well, although with a generally lower frequency than in the natural host *A. cerana*. (Fries et al. 1996). Recapping behaviour could be described as derived from hygienic behaviour. It involves the worker bees uncapping the brood cell, investigating the contents and later recapping the cell without removing the brood (Oddie et al. 2018). Higher frequency of this behaviour has been observed in several different naturally resistant communities in Oslo, Norway; Gotland, Sweden; and Avignon, France (Oddie et al. 2021). A study of all these populations compared to geographically similar populations treated for Varroa show a correlation with elevated recapping behaviour and lower mite reproductive success (Oddie et al. 2021). Note that this behaviour exists in susceptible honeybee populations too but has a higher efficacy in these reported naturally resistant colonies and, while a positive correlation to lowered mite reproductive success have been identified, the behaviour has not been proven as the cause (Oddie et al. 2021).

1.8.2 Grooming

Another trait common among bees is grooming, where adult bees identify parasites on itself or a hivemate and manually removes it. This behaviour follows the trend of being more prominent in Asian honeybees. One study found that over 99% of mites were removed from *A. cerana* colonies through grooming behaviour while *A. mellifera* colonies only removed 0.3% through grooming (Peng et al. 1987). So while Western honeybees exhibit this behaviour it is not nearly effective enough to provide resistance.

1.9 Aim of this work

This review aims to complement previous works and provide a complete synthesis of the state of bee colonies naturally resistant to Varroa. This synthesis will also provide a status of feral bee colonies that survive without treatment. It will also

provide an overview of traits that seem to allow the colonies to survive the mite infestations by presenting each presently known naturally resistant bee colony and discussing the mite-resistance traits adopted by that population. This information is presented in Figure 1 showing the status of each of the commonly shared resistance traits in all of the discussed resistant populations.

2. Results

2.1 Studied Naturally Surviving *A. mellifera* populations

2.1.1 The African honeybee in South America and South Africa

Varroa was first found in the Africanised honeybee *A. m. scutellata* in the early 1970s in Brazil (Goncalves and De Jong, 1981). The initial infestation rates recorded were very high, resulting in the Varroa invasion being taken seriously as it was thought to pose a large threat to South American apiculture (Morse & Goncalves, 1979). However, Brazilian bee colonies were not devastated by the mite, with later research showing that *A. m. scutellata* does not require mite control to survive infestation, which are often kept to a very low degree (3-4 mites/100bees) (Moretto et al. 1995). The initial high infestation rates followed by sufficiently self-controlled mite populations suggest that the host bees likely adapted to the parasite (Moretto et al. 1995). The Japanese haplotype was the first one to be observed in South America but have now been replaced by the more virulent Korean haplotype over almost the entire continent (Rosenkranz et al. 2010).

Hygienic and grooming behaviour have been found to be important traits providing resistance to the Africanized bees in both Brazil (Moretto, 2002) and in Mexico (Mondragon et al. 2005). While mite fertility has been observed to be as low as 50% in Africanized honeybees in Brazil (Rosenkranz, 1999) during the earlier years of the Varroa invasion, it has reportedly increased to more than 80%, probably owing to the change from Japanese to the more virulent Korean haplotype (Garrido et al. 2003). This increase in mite fertility has not greatly affected the stability of Brazilian *A. m. scutellata* populations, and no increased mite infestation rates have been reported (Garrido et al. 2003). This indicates that the mite resistance in the Brazilian population is not based on reducing the parasitic virulence of the mite, but rather to a combination of different resistance traits working in unison to limit infestation damage (Locke, 2016).

The mite was later discovered in South Africa in 1997 (Allsopp, 1997). Originally the mite was found to have similar reproductive success in *A. m. scutellata* as it had in *A. mellifera* when it first invaded Europe and the invasion was thought to cause a similar devastating effect on the African apiculture as it had in Europe (Martin & Kryger., 2002, Allsopp, 2006). Although colony losses did occur with the initial invasion of the mite the situation has since become stable, again suggesting a quick adaptive response from *A. m. scutellata* (Allsopp, 2006), much like what happened in Brazil. This level of resistance from the

African bees caused beekeepers to even be unaware of the mite infestations as Varroa spread throughout Eastern Africa (Muli et al. 2010).

The resistance of *A. m. scutellata* in both Africa and South America can be attributed to their already preexisting genetical traits, which would explain their fast adaptation (Locke, 2016). In addition to their parasitic resistance traits like grooming and hygienic behaviours, *A. m. scutellata* has several life history traits that may favour their survival from Varroa. These traits include high rates of absconding, migratory swarming, faster colony development and generally smaller colonies than their European counterparts (Fletcher., 1978, Moritz & Jordan., 1992, Schneider et al. 2004., Reviewed by Locke, 2016). When a colony swarms 40-70% of the adult worker bees leave the colony with a significant amount of phoretic mites, the colony then has a broodless period where mite reproduction is restricted (Wilde et al. 2005).

2.1.2 Island of Fernando de Noronha, Brazil

An isolated population of Italian honeybees (*A. m. ligustica*) was taken to the Brazilian Island of Fernande de Noronha in 1984, initially to provide plant pollinators and enable honey production (De Jong & Soares, 1997). This also provided the mainland beekeepers an isolated breeding population of European honeybees that are much less aggressive (De Jong & Soares, 1997). Queens from Italy were introduced to queenless Brazilian colonies from the mainland which were infected with Varroa. Despite Varroa infestation, mite control was not needed for over 12 years and the colonies were large, productive and gentler than their mainland counterparts (De Jong & Soares, 1997). The mite infestation rates were higher on the island than in the mainland as well as mite fertility which was above 80% while the mainland Africanized bees showed round 50% mite fertility at the time (Rosenkranz & Engels, 1994). Mite infestation rates was reduced from 26 to 14 mites/100bees in the five years between 1991 and 1996 probably caused by host adaptability (De Jong & Soares, 1997). Pairwise comparison done in Germany of these colonies by Correa-Marques et al. (2002) found that the colonies headed by queens from Fernando de Noronha showed significantly less grooming behaviour, and no differences in infestation rates from susceptible colonies could be found.

The survival of these bees cannot be attributed to hybridizing with Africanized bees as mitochondrial DNA analysis in 1996 showed all the sampled colonies were still 100% of the *A. m. ligustica* subspecies (De Jong & Soares, 1997). One possible explanation of the resistance is the mite haplotype; the colonies on the Island of Fernando de Noronha are only parasitised by the Japanese haplotype of the Varroa mite that arrived when the European bees were first introduced (Strapazzon et al. 2009). The isolation of this population has also been brough

forward as a possible explanation of their survival as it would limit the spread of honeybee viruses throughout the population. Brettel & Martin (2017) writes that the honeybee population of the island have evaded catastrophic consequences that Deformed Wing Virus would bring because of its very small and isolated population size, but that these factors only delay the development of a sufficient virulent version of the virus and does not stop it entirely.

2.1.3 Primorsky, Russia

The oldest known association between European honeybees and Varroa is from east Russia, where the older of the two documented host switches happened in the middle of the 19th century (Danka et al. 1995). When first examined, the European *A. mellifera* colonies found in the area seemed to resist the Varroa infestation, likely due to natural selection during their long time interacting with the mite (Danka, 1995).

Bees from the Primorsky region near Vladivostok were imported to USA during their initial testing (Rinderer, et al 1997). After inspection the bees were bred to support a commercial stock that had increased Varroa resistance which were released to commercial breeders in 2000 (Tarpy et al. 2014). The precise degree of resistance or exactly which traits enable the colonies resistance is not known for the bee stock. However, several resistance factors have been identified including, a reduced number of viable female offspring in the mite (de Guzman et al. 2008) and elevated levels of both hygienic (Kirrane et al. 2018) and grooming behaviour (Guzman-Novoa et al. 2012).

2.1.4 Avignon, France

During the 1990s, honeybee colonies that had been untreated for at least 3 years were sought out and collected in two different places in France: Avignon, and Le Mans (Le Conte et al. 2007). Wild colonies, such as from old, abandoned apiaries, and colonies reportedly untreated for at least 2 years against Varroa were all collected totalling 52 colonies in Avignon and 30 in Le Mans (Le Conte et al. 2007). Between the years 1999 and 2005 no significant differences in annual colony mortality could be found between these untreated colonies and treated mite susceptible colonies in surrounding area (Le Conte et al. 2007). The mite infestation rates were only about a third of untreated colonies in relation to close mite-susceptible colonies, suggesting the resistant bees had some way to limit Varroa's population growth, a costly one indicated by the differences in produced honey, with the resistant communities produced only about half as much honey (Le Conte et al. 2007).

One study found genetically increased olfactory cognition and neuronal activity in the resistant Avignon bees, traits generally higher in hygienic bees (Navajas et al. 2008). The study argues that olfaction and neuronal sensitivity together may play a major role in the detection of Varroa infested cells which in turn is a key element of hygienic behaviour (Navajas et al. 2008). Mite reproductive success was also lowered by 30%, probably owing to the adults more frequent removal of reproducing mites because of increased stress signals from brood being fed on by many newly hatched mites (Navajas et al. 2008., Locke et al. 2012). Elevated recapping behaviour has also been observed in Varroa resistant colonies from Avignon (Oddie et al. 2018a). The possibility of improved virus tolerance and/or resistance has been investigated as a cause for the French honeybee population's survival. When analysed, the resistant communities in France have shown to have less Acute bee paralysis virus and Chronic paralysis virus, though these bees did not survive longer than susceptible control bees when injected with both viruses (Le Conte et al. 2020). This suggests that the lower rate of virus in the population is an effect of lower rates of Varroa and not virus resistance.

2.1.5 Arnot Forest, USA

Thomas Seeley has been studying a population of feral honeybees nesting in trees in the Arnot forest, NY, USA since 1978. In 2002 Varroa had been present in the area for 15 years where he observed the colonies survival (Seeley, 2007). He then started a study over three years from 2002-2005 to determine the state of these feral bees and their relation to Varroa. This study revealed that the population of colonies in Arnot forest were stable despite the Varroa infestation and absence of treatment, and a comparison with susceptible control colonies showed no difference in mite infestation growth rate (Seeley, 2007).

The feral colonies tendency to nest in tree cavities that are generally smaller than hives was hypothesized to support mite resistance (Loftus et al. 2016). This was also supported by a later study analysing the life history traits of the feral bee colonies of Arnot forest both before and after the arrival of Varroa (Seeley, 2017). The results show that colonies from before the Varroa invasion (1970) and colonies after (2010) have almost identical life history traits (Seeley, 2017), suggesting that the feral colonies of the Arnot forest have adopted Varroa resistance traits that are efficient yet cheap enough to not affect their life history traits in a significant way. Seeley (2017) suggests that the natural life history of these bees, specifically their fast development, small colony size, and tendency to swarm often, provide them with some degree of resistance and could explain why these bees did not have to evolve new costly resistance traits. This could be

defined as the feral bees of Arnot Forest having some degree of “preadaption” to the mite infestation.

The possibility of adaptively decreased virulence of the DWV circulating in the population in Arnot forest contributing to honeybee survival have also been recently investigated (Ray et al. 2023). The results show that viral loads and infection rates were not significantly different between the feral bees of Arnot forest and managed control bees, but it was found that infections from isolates of DWV from Arnot forest usually resulted in milder symptoms and affected bees had higher chance of survival compared to isolated of DWV from managed control colonies (Ray et al. 2023). The authors speculate that this is initial evidence of a strain of DWV with relatively low virulence circulating in Arnot Forest (Ray et al. 2023).

2.1.6 Østlandet, Norway

A study by Oddie et al. from 2017 investigated a population of local honeybees from Østlandet in Norway, which had survived more than 19 years without Varroa treatment. The mite population and their reproductive success were investigated along with two common resistance traits: grooming behaviour and Varroa sensitive hygiene (VSH). While both grooming and VSH did not appear to be of particular importance for the population’s mite resistance, the infestation level was significantly lower in the resistant communities and the mite’s reproductive success was lowered compared to control communities, indicating it as a key factor (Oddie et al. 2017). Another factor that seemed to contribute to the survival of the colonies is their shorter postcapping period (Oddie et al. 2018b), which seemed to be a life history trait that naturally enables resistance to the mite, like the feral colonies of Arnot forest’s smaller hives and tendency to swarm.

This population has also been one of the several naturally resistant populations worldwide that show elevated levels of recapping behaviour (Oddie et al. 2018a). A Recent study in Sweden by Scaramella et al. (2023) analysed bees from three different naturally Varroa resistant populations found that average mite reproductive success in these Norwegian bees was not significantly lower than the control population of susceptible bees. Contrary to what was reported when previously inspected in Norway (Oddie et al. 2017). Scaramella et al. (2023) hypothesize that this might be caused by the population’s mite resistance traits being better expressed in their local environment, or that they have adapted specifically to the Norwegian mites.

2.1.7 United Kingdom

In the United Kingdom, bees that had not been treated for Varroa during recent years were collected from volunteer beekeepers between the years 2017 and 2019. These naturally Varroa resistant communities were then analysed in regards for two common factors of naturally resistant populations, namely, reduced mite reproductive success (MRS) and elevated recapping behaviour. The results showed that both elevated recapping behaviour and reduced reproductive success in the Varroa mite were traits that enabled naturally evolved long-term mite resistance (Hawkins & Martin, 2021).

The reproductive success of the mites was significantly lower in the Varroa resistant communities. The recapping behaviour was significantly more abundant in Varroa resistant communities and strongly targeted toward mite-infested brood cells. However, the results did not show a significantly lower mite reproductive success in the recapped cells (Hawkins & Martin, 2021), suggesting that, in these populations, recapping behaviour does not increase mite resistance through decreasing mite reproductive success in the recapped cells. The reduction of MRS was instead accounted to increased offspring mortality or underdevelopment, especially in the male offspring (Hawkins & Martin, 2021).

2.1.8 Cuba

European honeybees (*A. m mellifera*) were first introduced to the Caribbean Island of Cuba in 1768 from Florida, USA (Genaro, 2008). Varroa was first detected on the island in 1996, hypothesized to have entered through shipping of illegal queen import a couple years earlier (Perez & Demedio, 2017). In 1997 around 8000 colonies infested with Varroa died, and by 1998 the mite had spread throughout the island (Luis et al. 2020). After these initial losses caused by Varroa invasion, treatment for Varroa stopped and the honeybee population have now been free of treatment for over 22 years (Luis, 2020). The number of colonies present on Cuba is estimated at 220 000, this would make the Cuban population of European honeybees the largest Varroa resistant population in the world (Luis, 2022).

These resistant Cuban communities had a highly elevated recapping behaviour, where the average recapping rate of infested worker brood was 72%, compared to uninfested worker brood, which were recapped at 33% (Luis, 2022). This is in high contrast from Naïve colonies from Hawaii that had a mean recapping rate of 3,6% (Luis, 2022). Grindrod & Martin (2021) highlight that the resistance traits evolved in African subspecies (*Apis mellifera capensis* & *Apis mellifera scutellata*), European and Africanized honeybees (African x European hybrid) all appear to follow a trend. The Cuban Varroa-resistant population also follow this trend of elevated recapping behaviour, higher mite removal and reduced mite

reproductive (Luis, 2022). In this population however, elevated recapping behaviour was found to have no significant reduction of mite reproductive success in recapped cells (Luis, 2022), similar to the study of the UK population (Hawkins & Martin, 2021).

2.1.9 North County Dublin, Ireland

Varroa was first identified in North County Dublin in 2003 (McMullan, 2018). By 2010 it became evident that colonies where annual Varroa treatments had been missed, due to unfortunate weather conditions for example, showed less harmful effects of the mites compared to those with consistent Varroa treatment (McMullen, 2018). Since then, many beekeepers independently began treating their hives for Varroa less and less which resulted in a slow reduction of acaricide treatment (McMullen, 2018).

In an article released in 2018 beekeeper and writer McMullan reports that he had not treated his bees for the latest seven years and that by May 2017 almost two thirds of the beekeepers in the region had completely stopped their Varroa treatment. Smith et al. (2023) hypothesize that the less intense beekeeping in this area provided great conditions for the transition of the local population to Varroa resistance. They argue that the initial invasion of the mite could have potentially wiped out the local subspecies *Apis mellifera mellifera* if not treated by the beekeepers, but that the steady reduction of acaricide treatment over time allowed natural selection to favour Varroa resistance in the population (Smith et al. 2023). The number of mites falling from the hives suggested a high mite population in the 5 colonies studied by McMullen (2018), which he used to draw a parallel to the mite resistant feral bee population of Arnot Forest (Seeley, 2007) that also portrayed relative high mite fall values. The effects of Deformed Wing Virus were common in the population when Varroa had recently arrived, even at the start of treatment, and then again when beekeepers adopted the non-treatment approach (McMullen, 2018). However, McMullen reports that during his study between 2016 and 2017 signs of DWV were uncommon even though the relative mite populations were high (McMullen, 2018). Damage on the idiosoma of the fallen mites were more common during the 2016 study compared to 2004 and the beginning of Varroa infestation, at 3.5 and 2.0 times higher for mother and daughter mites respectively (McMullen, 2018). An increase of this degree indicates a large substantial reduction of mite reproductive success (Lodesani et al. 1996).

2.1.10 Gotland, Sweden

An isolated population of 150 honeybee colonies was established on the Swedish island of Gotland during the late 1990s, with many subspecies taken from various locations around Sweden (Fries et al. 2003). The colonies were then artificially infested with equal amounts of *Varroa* and left otherwise completely unmanaged (Fries et al. 2003). This experiment method was dubbed “Bond test” from the popular movie name “Live and let die”.

The individual colonies were then monitored for several different factors, including frequency of swarming, autumn mite infestation rates, winter losses and colony size in spring. After the first 3 years following the start of the experiment more than 80% of the colonies had died, with the leading cause being rapid buildup of mite population (Fries et al. 2003).

Swarming was frequent the first two years of the study but by the third year the colonies were weak and swarming rate was low (Fries et al. 2003). After these initial losses the autumn mite infestation rates and winter mortality decreased and swarming frequency stabilized again (Fries et al. 2006), with the mortality rate of colonies dropping below 20% six years after the start of the experiment (Fries et al. 2006).

A study from 2007 by Fries & Bommarco included a cross-infection experiment with control mite-susceptible bees showed that the Gotland bees had an 82% lower mite population growth rate. This indicated that the survival of the Gotland bee population is due to adapted mite-resistant host traits that had occurred through natural selection (Fries & Bommarco, 2007). The Gotland population have, in relation to mite-susceptible bees in a similar environment, smaller broodnests (Fries & Bommarco, 2007), a smaller population, fewer adult bees during the summer, about half the amount of worker brood and about one tenth the amount of drone brood (Locke & Fries, 2011). The Swedish resistant population have also displayed a significantly increased recapping behaviour targeted towards infested brood cells (Oddie et al. 2018).

Locke et al (2014) found that the resistant Gotland population has increased tolerance and/or resistance to several different honeybee virus. Notably for DWV, the study found that the resistant bees survived winters when the mite-susceptible bees did not, even though no significant differences in DWV titres was found between the mite resistant Gotland population and the mite-susceptible control population (Locke et al. 2014). A recent study from Svobodova et al (2023) regarding the gut microbiota of the *Varroa* resistant bee population on Gotland revealed that the population might have evolved to recruit beneficial bacteria leading to virus-tolerant assemblies. The study also brings forth the possibility of the bee population being colonized by novel symbionts that have driven host evolution (Svobodova et al. 2023).

2.2 Feral Honeybee Populations

There has been a consensus among researchers and beekeepers that, since the Varroa mite's invasion of Europe, colonies not treated against the mite will die within a couple years from direct and indirect damage from the infestation (Rosenkranz et al. 2010., Martin et al. 2012). This has led to the assumption of many that wild feral honeybee populations in Europe that have no human interaction have been all but wiped out (Moritz et al. 2007., Meixner et al. 2015). However, the example of the naturally resistant bees of Arnot forest studied by Seeley (2007) along with a study conducted in two different German beech forests found several feral honeybee colonies (Kohl & Rutschmann, 2018), showing that it is indeed very possible for unmanaged feral colonies to survive. The persistent survival of these feral colonies so long after the invasion of Varroa into the region would strongly indicate that natural selection has acted on them and lead them to develop Varroa resistant traits.

The German study of feral bees from 2018 found that in two different beech forest areas, the density of colonies were 0.13 and 0.11 colonies per square kilometre respectively (Kohl & Rutschmann, 2018). However, the estimated population density of these European forests is quite low compared to the temperate forests of New York State that show an estimated colony density of 1.0 colonies per square kilometre (Seeley, 2007). Similar findings of persisting feral bee colonies with a similar colony density have been made throughout Europe, including UK (Thompson, 2010), Poland (Oleksa et al. 2013), Ireland (Browne et al. 2020) and Spain (Rutschmann et al. 2022). However, a recent population demography study by Kohl et al (2022) of feral bees in German forests conducted over 4 years showed that only one of 10 feral colonies survived annually. They further found that colonies don't reproduce at the same rate, indicating the population is reliant on an influx of new swarms escaping from nearby apiaries to persist (Kohl et al. 2022). Kohl et al (2022) argue that all known self-sustaining feral colonies show much higher colony densities and that these feral populations listed above with relatively similar low population densities all likely follow the same trend of being sustained through an influx of colonies from apiaries. They also highlight in their results that bee colonies prefer nesting sites that have housed hives before, which could result in a new colony in the same hive being wrongly documented as the colony surviving (Kohl et al. 2022).

An article released in 2021 by Bila Dubaić et al. brings to light a very particular case in the Serbian capital of Belgrade where supposed feral colonies have been repeatedly reported all over the populated urban area. The study utilizes a dataset compiled from reports of bee colonies and swarms from citizens, usually from calls to ask for their removal (Bila Dubaić et al. 2021). Bila Dubaić et al. (2021) states that beekeeping is almost non-existent in the urban core and that managed apiaries are relatively rare even in the areas surrounding the urban

environment. They draw the conclusion that it is unlikely the number of colonies recorded are caused by escaped bees from surrounding apiaries and expect >90% of the occurrences in this dense urban area to be essentially feral (Bila Dubaić et al. 2021). In Belgrade, several colonies were observed to survive for several years further supporting the author's hypothesis that the bee population in Belgrade is feral and thriving (Bila Dubaić et al. 2021) without depending on an influx of new colonies escaped from apiaries but rather a self-sustaining population, that have, through natural selection, adapted to survive Varroa infestation (Bila Dubaić et al. 2021). The specific interactions of this urban feral bee population with Varroa have not been studied in detail and their method of survival remains to be seen.

2.3 Citizen Science: The future of Varroa research

Citizen science is defined as relying on the active involvement of the public in the provision of data and creation of scientific knowledge (Miller-Rushing et al. 2012). The study of the feral bees in Belgrade utilizes a form of citizen science. The environment of the study is densely populated, and records of colonies and swarms were plentiful since they were often in the close vicinity of people's homes, conditions that are great for citizen science (Bila Dubaić et al. 2021). Moro et al. (2021), highlight the benefits the use of citizen science could have in identifying potential cases of *A. mellifera* naturally surviving Varroa infestation without the intervention of beekeepers or researchers. Citizen science can be an effective tool in tracking the conservation status of free-living honeybees (Moro et al. 2021), and to build datasets of such wild populations, much like the Belgrade case (Bila Dubaić et al. 2021).

With the use of a 20-question survey released in 2018, Moro et al (2021) collected data of 241 managed colonies and 64 free-living colonies from 28 different colonies. Their data suggest that there could be twice as many naturally surviving colonies worldwide than are currently known (Moro et al. 2018). Even though the data from these surveys need to be confirmed their results show how citizen science can be a powerful tool for future bee research, increasing generated data, broadening opportunities for comparative studies and fostering collaborations between beekeepers, scientists and citizens (Moro et al. 2021).

3. Discussion

The findings from this study provide insight into the natural resistance against Varroa mites in various *A. mellifera* populations worldwide. These findings suggest that the resistance observed in these honeybee populations is a result from a combination of behavioural traits, life history traits and potential local adaptations to the mites and their environment.

3.1 Behavioural traits

Hygienic behaviour along with the more suited Varroa sensitive hygienic behaviour are crucial aspects of the resistance mechanisms observed. Varroa sensitive hygiene in particular involve detecting and removing mite-infested brood from the hive, effectively reducing the mite population and limiting the spread of associated diseases. In resistant populations, such as those in the UK and Cuba, bees exhibit enhanced abilities to identify and remove mite-infested brood compared to non-resistant populations (Luis, 2022., Richards & Hawkins, 2021). This action disrupts the mites' reproductive cycle and significantly reduces their population within the colony.

Associated to hygienic behaviour is recapping behaviour, several of the documented resistant bee colonies often uncap and recap brood cells (Luis, 2022., Richards & Hawkins., 2021, Oddie et al., 2021, Oddie et al. 2021). The amount of resistant bee populations exhibiting this behaviour indicates it is related to mite resistance although no precise description of the resistance inducing mechanism could be found.

In addition to hygienic and recapping behaviour, grooming behaviour play a significant role in Varroa resistance. Elevated grooming behaviour, where bees remove mites from themselves or their hive mates have been observed in several of the Varroa-resistant populations worldwide, including *A. m. scutellata* in Brazil (Moretto, 2002), Primorsky (Guzman-Novoa et al. 2012) and Avignon (Navajas et al. 2008). This is not surprising as grooming behaviour is one of the traits that Varroa's natural host *A. cerana* have adapted to combat the mite infestation.

Another side of Varroa resistance is life history traits that help reduce Varroa infestation. The feral colonies of Arnot forest along with *A. m. capensis* in South Africa and the Africanized bees of Brazil all exhibit higher swarming rates, smaller colony sizes and rapid development, which all seem to help maintain low levels of infestation (Fletcher., 1978, Moritz & Jordan., 1992, Schneider et al. 2004., Reviewed by Locke, 2016. Seeley, 2017).

Reduced mite fertility is a noteworthy aspect of resistance reported in several mite-resistant populations including, France (Navajas et al. 2008), Østlandet

(Oddie et al. 2017), United Kingdom (Hawkins & Martin, 2021), Cuba (Luis, 2022), and Ireland (McMullen, 2018). However, a universal cause for the reduction in mite reproduction in all these resistant communities have not been identified.

Local adaptations and environmental factors also play a vital role in shaping resistance mechanisms. Norwegian bees, for instance, show different levels of mite reproductive success when studied in their local environment compared to other settings (Scaramella et al. 2023), suggesting adaptation to specific mite populations and environmental conditions. Similarly, Africanized honeybee populations in Brazil maintain stability despite the presence of the more virulent Korean haplotype of *Varroa* (Garrido et al. 2003), indicating robust resistance mechanisms not solely dependent on mite virulence.

Honeybee virus tolerance is another important component of *Varroa* resistance. *Varroa* not only weakens bees directly through feeding on fat bodies, but also vectors several harmful viruses, including DWV. Several mite-resistant bee populations have traits or specific situations that alter their relationship with these viruses, including the isolation of the bees on Fernando de Noronha which hinder a too virulent virus strain to emerge (Brettel & Martin, 2017). The strain of DWV in the feral bees of Arnot forest that causes milder symptoms increases the survival rate of affected bees (Ray et al. 2023). Lastly a supposed tolerance of DWV in Gotland bees lets them survive winters with amounts of virus that usually kill other mite susceptible bees (Locke et al. 2014).

This paper has found that the traits allowing honeybee communities to be *Varroa* resistant are not universal, but do follow a trend, with a few traits being adopted in several different population across the globe. A recent paper by Grindrod & Martin (2021) came to a similar conclusion where they highlighted mite-resistant honeybee populations tendency to adapt recapping behaviour, brood removal and mite infertility and present a framework to link these traits together (Grindrod & Martin, 2021). A complete summary of the resistance traits in these populations are given in Figure 1

	BRAZIL	SOUTH AFRICA	FERNANDO DE NORONHA	PRIMORSKY	AVIGNON	ARNOT FOREST	ØSTLANDET	UNITED KINGDOM	CUBA	IRELAND	GOTLAND
HYGIENIC BEHAVIOR	YES	YES	NO	YES	YES		NO		YES		NO
RECAPPING BEHAVIOUR					YES		YES	YES	YES		YES
GROOMING BEHAVIOUR	YES	YES	NO	YES			NO		YES	YES	NO
SMALL COLONY SIZE	YES	YES	NO	NO		YES					YES
REDUCED DEVELOPMENT TIME	YES	YES					YES				
REDUCED MITE REPRODUCTION	NO	NO	NO	YES	YES		YES	YES	YES	YES	YES
VIRUS INTERACTION			YES		NO	YES					YES

Figure 2. A grid of the most common resistance traits documented in these *Varroa*-resistant populations. Green and YES if the population exhibits the resistance trait, red and NO if no difference has been found from susceptible population and blank if the trait has not yet been investigated.

3.2 Conclusion

Several populations of *A. mellifera* that are resistant to the mite have appeared worldwide. These mite-resistant communities and the traits that allow them to survive the *Varroa* infestation can act as examples for industrial breeding to produce a *Varroa* resistant stock. While many of the resistant populations and the traits they have developed follow the same trend, having many similar traits that provide their resistance, all of them do not. Some populations completely lack traits that have been observed to be a key feature for other. The resistance of some populations have diminished when exposed to new environments, suggesting some resistances may be dependent on the local environment or ecosystem. Differences between these mite-resistant populations, both geographical and environmental, show that resistance can be attained everywhere. Differences in the traits that the populations have adopted show that resistance can be attained through a row of different methods.

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