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Varroa destructor: A complex parasite, crippling honeybees worldwide

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Abstract

The parasitic mite, *Varroa destructor*, has shaken the beekeeping and pollination industries since its spread from its native host, the Asian honeybee (*Apis cerana*), to the naïve European honeybee (*A. mellifera*) used commercially for pollination and honey production around the globe. Varroa is the greatest threat to honeybee health. Worrying observations include increasing acaricide resistance in the varroa population and sinking economic treatment thresholds, suggesting that the mites or their vectored viruses are becoming more virulent. Highly infested weak colonies, popularly dubbed “mite bombs”, facilitate mite dispersal and disease transmission to stronger and healthier colonies. Here, we review recent developments

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in the biology, pathology and management of varroa, and unearth old knowledge that was lost in the archives.

A Formidable Foe

Remarkably adaptive and complex (**Figure 1, key figure**), *Varroa destructor* [1] (hereon referred to as varroa, unless otherwise stated) is linked to the worldwide decline in honeybee (*Apis mellifera*) health [2]. The global spread of varroa has been assisted by international trade (**Box 1, Figure 2a**) [3], and while numerous mitochondrial **haplogroups** have been defined, the Korean K1 is the most pervasive (**Figure 2b**). No other pathogen or parasite has had a comparable impact on honeybees, in part because varroa only recently adapted from its original host, the Asian honeybee (*A. cerana*) (**Figure 3**) to exploit a naïve host with inadequate innate defenses. Varroa incurs only limited damage to *A. cerana* colonies due to several host defense mechanisms that impact varroa reproduction: mite infertility in worker brood [4], entombment of drone brood infested with multiple mites [5], and increased hygienic behavior [6]. The recently re-updated varroa genome (GCA_002443255.1) [7] will be a powerful tool to help understand varroa evolution in response honey bee novel defenses traits, host-switching, and successful global invasion.

Varroa mites are “wingless, eyeless, and unable to crawl between widely spaced honeybee nests” [8]. Yet monitoring efforts show that honeybee colonies are almost universally infested [2]. Colonies often experience unnatural surges of varroa when nearby colonies collapse [9], potentially due to drift, and definitely due to robbing, when bees from healthy colonies exploit poorly defended, collapsing colonies to steal honey [8]. These varroa-laden, collapsing colonies are often referred to colloquially as “mite bombs.” Furthermore, varroa is a dangerously efficient vector of several bee viruses, which has dramatically worsened the virus landscape [10]. We have underestimated varroa’s adaptive ability: the mite has expanded its host range multiple times, has excellent chemosensing abilities [11], engages in chemical mimicry [12], and manipulation of its host [13], readily disperses within and between colonies [8, 13], engages in parental care [14], and rapidly evolves acaricide resistance [15]. Apicultural practices create a

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virtually limitless supply of new host colonies. Most colonies are treated prophylactically with acaricides, limiting natural selection's ability to improve host fitness against this parasite. However, there are signs in isolated *A. mellifera* populations that a host-parasite equilibrium can be achieved.

Understanding the Varroa Life Cycle

The life cycle is split into two distinct phases:

- the reproductive phase that takes place inside honeybee brood cells, where a **foundress** mite raises her young
- the **dispersal phase**—often incorrectly termed the **phoretic phase**—where mature female mites travel and feed on adult bees.

Reproductive Phase

In *A. mellifera*, varroa typically produces 0.7-1.45 mature female daughters in worker brood cells [16] and 1.6-2.5 daughters in drone cells [17]. Varroa use **kairomones**, a form of "chemical espionage" to invade appropriately aged larval cells [18] (**Figure 4a**), exhibiting an 8-fold preference for drone brood, where they have increased reproductive potential [19]. Relative proportions of worker and drone brood expand and contract throughout the season, and, therefore, so does the varroa population (**Figure 4b**). Inside the brood cell, the foundress hides, immobile, in the pool of food at the base of the cell, breathing through her raised **peritreme** that extends above the liquid food like a straw [20]. This immobility may be an adaptation to minimize removal by varroa-sensitive worker bees, as prior to and during capping, nurse bees frequently inspect the cell. After cell capping, the honeybee larva finishes the brood food, stretches out along the length of the cell and spins a cocoon. During this final larval feeding bout, the mite leaves the brood food, climbs onto the bee pre-pupae, and punctures a relatively large hole (100 µm) in the bee's cuticle to create a feeding site for herself and future offspring [21]. This feeding site remains open due to anticoagulants in mite saliva and suppression of host wound healing [22].

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Feeding on the larval fat body is a prerequisite for varroa reproduction [23]. Signals from the bee larvae trigger mite reproduction [24] and influence the gender of the egg [25]. Sex determination in varroa is via **arrhenotokous parthenogenesis**: males are haploid with seven chromosomes, while females are diploid with 14 chromosomes. However, it remains unknown if sex determination in varroa involves a distinct genetic sex-determination locus, as is the case in honeybees and other hymenopterans.

Initiating Oviposition

Initiating oviposition is an energetically demanding task, and foundresses derive this energy by metabolizing consumed honeybee tissue. Proteomic [26] and transcriptomic [27] studies identified a drop in carbohydrate metabolic enzymes during foundress egg-laying, whereas they are upregulated before and after egg-laying [27]. This finely tuned expression pattern matches remarkably well with periods of unmetabolized nutrient transfer from the host [28] and the foundress [29] to the egg. In essence, varroa eggs contain host (bee) proteins that avoided digestion and passed through the foundress mite untouched by catalytic enzymes. The foundress also requires some larval proteins and hormones (*e.g.* ecdysone) to initiate egg-laying [28, 30]. We speculate that foundresses sequester host molecules in their eggs, whereas peak enzyme abundance before and after oviposition fuels the energetic demands of egg production. Together, these observations paint a complex picture of nutrient transportation and sequestration from bee tissue through the foundress to her eggs.

The foundress deposits the first haploid egg approximately 60-70 h after cell invasion. The foundress engages in careful parental care, gluing the male egg to the upper cell wall (the safest spot during bee pupation) to ensure that the male protonymph can walk away after hatching [14]. Male mites often die during host pupation, due to pupal movement and the pupal legs blocking his access to the communal feeding site [31]. The foundress lays an additional diploid egg every 30 h thereafter, depositing them further down the cell wall. When the first female matures, she mates with her brother on the communal fecal pile (**Box 2**). Males mate almost exclusively with freshly molted females, and stop mating with older females when a younger

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one finishes her last adult molt [32]. If the male is dislodged or dies, the females emerge unmated. Formerly, it was believed these unfertilized varroa were unable to mate once they leave the cell, thus never producing viable offspring, not even haploid males. New research demonstrates that virgin varroa females can lay parthenogenic haploid eggs and then mate with their son, producing viable daughters under laboratory conditions [33]. Mites do not always initiate oviposition after cell invasion [34], potentially due to disruption in chemical communication [30]. Indeed, oviposition can be experimentally disrupted by applying (Z)-8-heptadecene to brood cells before capping [35], and other compounds can disrupt host-seeking behavior [36].

Under laboratory conditions, a female mite can have up to seven reproductive cycles during her lifetime and lay up to 30 eggs [37]. At the time she first matures, she receives 30-40 spermatophores via multiple matings with the male(s) in the cell, which she stores and uses during her lifespan [38]. In worker brood, a foundress with seven reproductive cycles would theoretically produce ~ 9 mature daughters, or ~ 18 mature daughters in drone brood. However, under field conditions it is estimated that each mite has 1.5 to 3 reproductive cycles [39].

Dispersal Phase

When a parasitized honeybee emerges from its cell, it carries the mature female mites (mother and daughters) from the cell. The daughter mites frequently switch to a nurse-aged bee [13] to activate their ovaries, allow the spermatophores to mature [38], and feed on adult bees. It was long believed that varroa was a tick-like parasite, feeding on hemolymph. However, varroa's mouthparts and digestive system are structured like an organism that feeds on semisolid tissue via extraoral digestion [40]. Varroa waste consists predominantly of guanine with traces of hypoxanthine, uric acid and caffeine [41], suggesting a protein-rich diet with limited water. New research overturned the decades-long belief that varroa feeds exclusively on hemolymph, demonstrating that the protein-rich fat-body is required for varroa egg production [23] and that stained fat body tissue was consistently detected inside the gut of mites feeding on honeybee

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adults [42]. Inspection of varroa feeding sites reveals a feeding hole between the overlapping abdominal plates of the honeybee and degraded fat body cells beneath the intersegmental membrane, likely due to extra-oral digestion from mite saliva toxins [42].

Parasite-Induced Changes Enable Colony Dispersal

Varroa mites alter the hydrocarbon cuticle of their hosts and adapt their preference for different adult host bee life-stages based on colony-wide varroa infestation levels [43]. At low mite abundance, varroa preferentially parasitizes nurse bees, which frequently tend to brood and thus provide many opportunities to infest an appropriately aged larval cell. Varroa distinguishes nurse bees from foragers by different chemical cuticular signatures [44]. When mite abundance increases in the colony, the chemical profile of nurses and foragers tends to overlap, promoting mite departure by dispersing onto foragers [43]. Parasitized brood develops into adult bees that spend less time nursing and mature at an accelerated rate [45]. These workers thereby contribute less to colony productivity, and potentially promote varroa dispersal to new colonies [1, 46].

Virus Transmission

By feeding on bee tissues, varroa acts as an efficient vector of pathogens between bees. Vector-based disease transmission involves three main phases:

- **Acquisition:** varroa feed on bee tissues, ingesting the pathogens that reside in those tissues
- **Mobility:** varroa moves freely between different individual hosts
- **Transmission:** during feeding, varroa introduces either secretions or partial gut content into the new host to complete the transmission.

Efficacy of vector-mediated virus transmission depends on a range of secondary conditions, such as what pathogens are present where the mite is feeding, pathogen survival between mite acquisition and transmission, the susceptibility of the receiving host, and whether or not the pathogen also replicates in the mite (biological vector) or not (mechanical vector) [47, 48]. These

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conditions differ significantly between individual viruses, impacting their virulence and their relationship with varroa [46].

Viruses Associated with Varroa

Many viruses have been detected in honeybees, with new viruses constantly identified (**Figure S1)** [49, 50]. Nearly all honeybee viruses can be efficiently propagated (amplified) by injecting them into pupae or adult bees [51] similar to how varroa feeds on its host. In theory, therefore, all of these viruses can be transmitted by varroa, but in practice only Deformed wing virus (DWV) and Acute bee paralysis virus (ABPV) have a clear vector relationship with varroa [52, 53]. Both DWV and ABPV have several major co-circulating variants that differ in virulence characteristics [47, 48, 52, 54-56]. Sacbrood virus (SBV) does not seem to be transmitted directly by varroa but keeps popping up as a co-factor in natural varroa resistance/survival and virus adaptation [57], DWV-induced bee mortality [58], general virus-host interactions and immunity [59], inter-virus competition [54], and varroa behavior [60]. SBV induces pollen aversion in bees and has therefore a strong effect on nursing, division of labor, foraging and bee nutritional status [51, 61], which themselves play major roles in varroa-virus virulence [45]. For other viruses, the relationship with varroa is indirect or non-existent [62, 63], even if varroa-mediated transmission can be demonstrated experimentally. The most extreme example of this is Slow bee paralysis virus (SBPV), which can be transmitted by varroa at both individual and epidemic level [64], but whose natural prevalence in honeybee colonies remains marginal [63].

Many bee viruses use several different modes of transmission, each with its own virulence rules and needs, allowing different virus variants with different properties to co-exist simultaneously [47, 48, 55, 56, 58, 65]. Host range and geographic isolation are two other common sources of major virus variants [53], which can spread through the global trade in bees (**Figure 2**). Major variants vastly increase the genetic options for the virus to adapt and change virulence. This is in part because coexisting virus variants can act cooperatively, sharing and exchanging their strongest features for mutual benefit. For example, DWV-B replicates efficiently in varroa [47], which means that DWV-A (which can't replicate in varroa [48]) can gain the replicative function

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when co-circulating with DWV-B [66]. If the roles are reversed in other infection scenarios, both variants gain from their cooperation. Recombinant viruses, where variants exchange whole genome sections, can be particularly virulent [55], because they combine the strongest parts of the variants into a single genome, no longer relying on cooperation for increased fitness or virulence.

Virus Symptoms and their Significance

Apart from the secondary factors mentioned above, colony mortality (in particular, the timing of this mortality during the bee season) strongly influences the real-world relationship between varroa and individual viruses [46, 67]. As an obligate parasite, varroa survival is intimately coupled to the availability of colonies, and beekeeper management often guarantees a limitless supply. DWV infection and symptoms (dwindling colonies and flightless bees) peak during autumn, when the nectar flows have ceased and strong colonies rob honey from weak colonies, providing a timely opportunity for varroa (and DWV) 're-invasion' into a strong colony immediately prior to overwintering [1]. The colony-level symptoms of DWV, plus seasonal timing, are therefore important features of varroa survival and transmission between colonies [46, 68]. The colony-level symptoms and mortality associated with varroa-vectored ABPV infections occurs earlier in the summer [67], prior to the robbing season, hence missing the opportunity to transfer varroa into colonies capable of overwintering [46].

Viral infection can also impair honeybees' social immunity defenses. For example, DWV-infected honeybees are less able to differentiate between varroa-infested and non-infested pupae [69]. Moreover, ABPV-infected pupae are efficiently removed by **varroa-sensitive hygienic** (VSH) behavior **(Figure 1d**), while DWV-infected pupae are not [70], which favors varroa-mediated transmission of DWV over ABPV. Similarly, hygienic bees preferentially identify [47] and remove [71] pupae infected with the more virulent DWV-B variant, thus helping the less virulent DWV-A variant persist in the population. Additionally, ABPV's higher virulence results in higher colony winter mortality (and thus varroa death), allowing for the gradual displacement of ABPV by DWV in varroa-infested colonies [62]. Overall, varroa is both vector and host for certain bee viruses,

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particularly DWV-B, and is also behaviorally affected by both DWV and SBV [60], adding a whole new range of factors to the dynamic between varroa, bees and viruses.

Virus Ripple Effects in New Territories

Arrival of varroa into new territories profoundly impacts the health of the bees and adds significant financial costs to beekeepers. For example, varroa first reached New Zealand on the North Island in 2000 [72] and spread until all regions of the country were impacted by 2013. This clearly delineated wave of spreading varroa provided a unique opportunity to compare virus expression between parasitized and non-parasitized colonies. The initial arrival of varroa led directly to a 16% drop in colony numbers [73]. New Zealand exemplifies the dramatic impact varroa has on viral virulence in its host. The rapid spread of varroa across the mainland of the country—less than 15 years to cover a 1,600-km territory—was accompanied by a dramatic change in the viral landscape, with each virus responding in a unique way [62].

Varroa's clear role in spreading the more virulent strains of different viruses was repeated in the Hawaiian archipelago [52]. The dynamic shifts in the observed viral titers suggest that the multiple viruses in honeybees interact to create a changing pathological landscape that peaks soon after varroa arrival (2-3 years for KBV, SBV and BQCV), before becoming more stable and predictable depending on the level of varroa infestation [62, 74]. However, DWV dynamics, regardless of varroa infestation, demonstrate escalating titers that continue to grow the longer the duration of varroa infestation, maintaining the DWV epidemic [62].

Viruses, Varroa Thresholds and Virulence Management

If virulence is not punished, it will proliferate. Keeping weak colonies alive during winter, either through sharper treatment thresholds [75] or by combining them with strong colonies, encourages the transmission and survival of virulent varroa and virus traits, much like re-invasion does. One of the most important, and least understood, practices in virulence management is culling, which is largely absent in beekeeping, other than for **American foulbrood**. Moreover, since the only host for varroa is the honeybee, which is overwhelmingly controlled by

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beekeepers, culling would be particularly effective for removing both inadequate honeybee genetics and virulent varroa-virus traits.

Social Immunity

In comparison to varroa's original host (*A. cerana*), *A. mellifera* has fewer individual behavioral defenses against the mite, the most prominent being grooming, hygienic behavior and varroa-sensitive hygiene.

Social Apoptosis

A. cerana worker brood is, perhaps counterintuitively, highly sensitive to a toxic protein secreted by varroa upon feeding. This "social apoptosis" limits varroa reproduction to drone larvae [76, 77]. This sacrifice has the dual effect of both disrupting the mite's reproductive cycle, as well as producing higher levels of larval decay as a theoretical stimulus for adult workers to perform hygienic behavior [76]. This varroa toxic protein does not have the same lethal impact on *A. mellifera* worker bee larvae, thereby increasing manifold the amount of suitable brood for mite population growth [77]. New research shows that brood-related traits in *A. mellifera* could be contributing to hygienic behavior, VSH, and the suppression of mite reproduction [78, 79]. Brood frames transplanted from non-hygienic to hygienic hives and *vice versa* produced hygienic scores correlated with the donor colony, rather than the recipient colony. Infested brood that is uncapped and removed by VSH bees is also developmentally delayed compared to non-targeted brood [70]. While it is currently not known if this brood effect is a widespread phenomenon in *A. mellifera*, it could be an evolutionary remnant of the drastic social apoptosis strategy observed in *A. cerana*.

Grooming

Allo- and auto-grooming contribute to varroa resistance by both removing mites from adult bees and by physically damaging the mites, preventing them from seeking a new brood cell to infest [80]. Honeybees can initiate allo-grooming via a "grooming invitation signal" – a whole-body vibrational dance lasting several seconds – which stimulates other workers to groom the

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dancer. Grooming workers use their mandibles and forelegs to forcefully remove the mites from adult bees, leading to mite injury or death [81]. In the United States, scientists have produced a strain of bees, now commercially available, that exhibit elevated grooming and mite biting [82].

Hygienic Behavior and Varroa-Sensitive Hygiene

Hygienic behavior (**Figure 1d**) is one of the best-studied social immune defenses, but our understanding of this trait is still limited. Olfactory cues released from dead, diseased, or parasitized brood are thought to diffuse through the cell cap and stimulate adults to perform the behavior. New odorants linked to hygienic behavior include oleic acid, tritriacontene, heptacosene, and components of brood ester pheromone [70, 83, 84], but contrary to conventional wisdom, all of these molecules are relatively non-volatile. Therefore, we speculate that either hygienic workers are extremely sensitive to miniscule amounts of these vaporized compounds, or workers may periodically open and inspect brood cells, looking for contact cues.

Hygienic removal of varroa-infested cells (also called **varroa-sensitive hygiene,** or VSH) is a specific subcategory of hygienic behavior that can only be partly predicted by general hygienic behavior tests such as the **freeze-killed brood assay** [85]. VSH-specific brood effects reduce mite fecundity [70, 84], and high-VSH colonies preferentially remove brood infested with multiple foundresses [86] or foundresses carrying highly virulent viruses [70] or DWV strains [47, 71]. Removing multiply-infested cells has the added benefit of deterring varroa outcrossing, potentially inhibiting the more virulent mites from spreading genetic material like acaricide-resistance (**Box 2**). Likewise, preferential removal of varroa carrying highly virulent viruses or DWV strains may set a virulence-limiting ceiling, helping to establish a new host-parasite equilibrium.

Genetic Foundation of Hygienic Behavior, VSH, and Grooming

There have been a large number of gene differential expression studies that have analyzed transcript and protein profiles associated with hygienic behavior, VSH and to a lesser extent

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grooming (reviewed in [87]). Some consistent trends include the differential regulation of odorant binding proteins, genes in the cytochrome P450 (CYP450) superfamily, and genes involved in biogenic amine chemoreception [87]. The differentially expressed genes are not always regulated in the same direction and there is a disconcertingly low degree of congruency among the differentially regulated genes identified by different researchers, but almost all have generally concluded that olfaction, neural signal transduction, and ligand degradation are key molecular processes underlying hygienic behavior and VSH [87]. This suggests that in addition to phenotypic plasticity – that is, one genotype giving rise to many phenotypes – varroa resistance mechanisms may also be presenting a degree of **genotypic plasticity**, *i.e.*, different biochemical pathways (with presumably different underlying genetic control) that ultimately result in similar phenotypes. However, these are mostly behavioral traits, and thus also subject to colony-level dynamics and their internal and environmental drivers, which could be further sources of poor congruency.

Honeybee Populations Surviving with Varroa

When varroa first arrives to new regions, it typically wipes out the majority of the feral colonies within a few years. Yet after the initial wave of losses, feral populations often reappear and persist [88].

Developing Varroa Resistance Without Human Intervention

Evidence of adaptation can be seen in several subspecies, including *A. m. scutellata*, *A. m. capensis* and multiple populations of Western honeybees (ssp. *carnica*, *mellifera* and hybrids) (reviewed in [88]). In contrast to active selection via controlled breeding programs, natural selection gears adaptive change towards host-parasite equilibrium within the context of the local environment. Populations of surviving bees found within Europe and North America were very likely founded by feral colonies that characteristically had little management, and resistance traits persisted through the genetic bottlenecks of progressive die-offs. The remaining colonies consistently presented varroa, but showed a lower colony mortality rate when compared to sympatric control colonies [88].

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Conserved Traits in Naturally Resistant Bees

Varroa resistant populations share multiple traits that permit survival despite parasite infection (reviewed in [87]). The Gotland "Bond bees" and the Arnot forest bees have consistently smaller colony sizes than commercial control stocks and a greater tendency for swarming [89, 90], and the Gotland and French populations both display reduced varroa reproductive success [91]. Evidence for VSH and mite-targeted grooming in these naturally-adapted populations has so far been mixed [92-94]. Despite adapting independently, the surviving populations seem to share a handful of common traits that work additively and permit prolonged survival [88, 95]. Often these survival traits are not well-aligned with the needs of commercially managed stock, where large populations, early and prolonged brood rearing, and no swarming are prized. One of the adaptations of naturally varroa-surviving honeybee populations is a highly elevated rate of re-capping behavior, which may disrupt varroa-reproduction without incurring social apoptosis, thereby significantly reducing the colony-level cost of natural varroa defense and improving the probability of colony survival [95].

Breeding Commercially-Viable Resistant Stock

A few commercial beekeepers have stopped chemical varroa interventions and continued to select for commercially desirable traits like honey production in their bees in France [96] and Norway [93]. Commercially-viable honey-producing stock since before the arrival of varroa, these populations display the same reduced mite reproductive success seen elsewhere [95], potentially due to ecdysone disruption in the bees [30] (which varroa requires for reproduction but cannot biosynthesize) or due to interruptions in the reproductive cycle by behaviors like brood removal and cell recapping. Some scientists have called for new methods of bee breeding that do not involve regular acaricide treatment, advocating that by increasing selective pressure, natural selection will evolve host-parasite equilibriums [97]. However, because of horizontal parasite transfer [8] this could threaten the livelihood of many beekeepers in the vicinity.

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An integrative method, involving treatment by necessity and selective queen rearing is recommended for those attempting to breed varroa-resistant bees. While field assays for measuring varroa resistance traits, like the VSH and grooming tests, are prohibitive for large-scale testing by beekeeping operations, **marker-assisted selection** via genetic or proteomic testing has been demonstrated to be economically viable [98]. However, if the speculated genotypic plasticity described above is occurring, that would mean that different honeybee populations may have different genetic or expression markers, which would complicate the utility of this approach. This hypothesis remains to be tested, which will be an important step for determining the usefulness of this technique on a large geographic scale.

Concluding Remarks: The Looming Threats of Other Mites

We are still learning about varroa and how to control it sustainably (see **Outstanding Questions**), but new RNAi techniques that inhibit varroa reproduction may help in the future [99]. Additional insight into varroa's basic biology, genetic architecture and demographic history are necessary to develop sustainable control measures and resistance breeding programs. The recently updated varroa genome [7] is a step toward leveraging population genomics and to understand varroa diaspora success despite reduced genetic diversity. But we should simultaneously prepare for two other mites that may soon be spreading worldwide. The cryptic *V. jacobsoni* has already switched hosts multiple times and could already be following the same path as *V. destructor* – a path that we urge researchers to track using DNA barcoding.

Another parasitic mite with multiple species, *Tropilaelaps* spp., has shown similar patterns, shifting from its original host, the Giant honeybee (*Apis dorsata*) to *A. mellifera*. Similar to varroa, currently only two of four species (*T. mercedesae* and *T. clarae*) parasitize the European honeybee [100], with *T. mercedesae* exhibiting a wider geographical distribution, which is still limited to East Asia. Rapid global trade and global warming could easily permit the wider distribution of *Tropilaelaps* to all regions inhabited by *A. mellifera*. Its biology and life cycle are poorly understood, making it difficult to develop approaches for management and control. Formic acid, thymol and chemical acaricides used for varroa treatment are being adapted for

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use against Tropilaelaps. Nevertheless, urgent research on how this pest has adapted to its new host is critical.

In South East Asia, co-infestation of *V. destructor* and *T. mercedesae* is common, and *Tropilaelaps* mites often outcompete varroa [101]. Previous research reports that *Tropilaelaps* has many of varroa's hallmark symptoms: it reduces host lifespan, lowers adult bee emergence weight, and promotes higher rates of wing deformity and higher DWV levels [102]. Experience with varroa and its rapid spread through globalization (**Figure 2, Box 1**) suggests that range expansion of *Tropilaelaps* is only a matter of time and countries should prepare for its arrival.

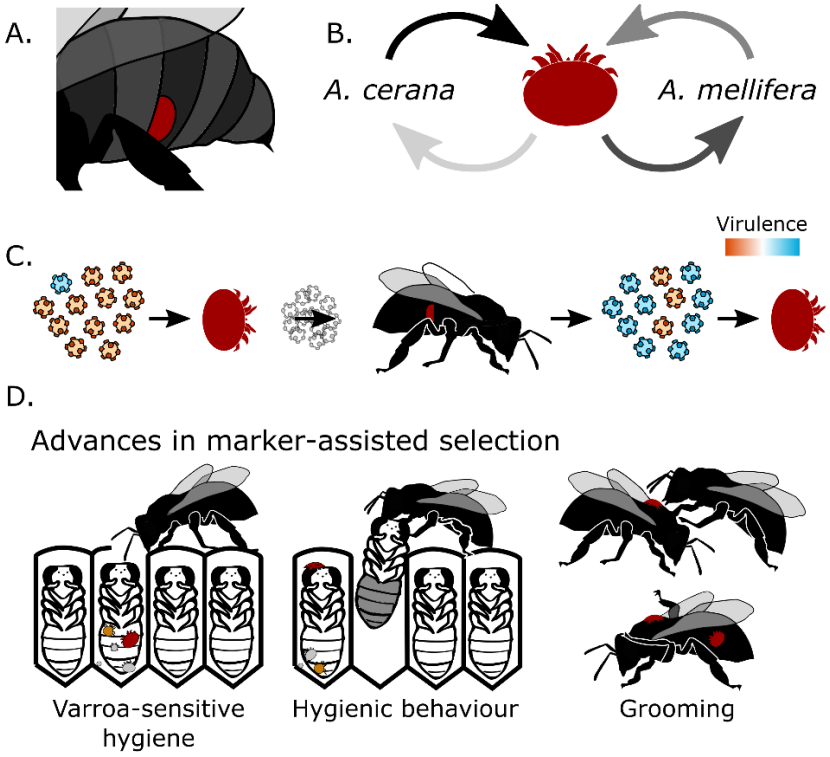

Figure 1. Advances in our understanding of Varroa destructor. In this review we examine varroa's biology, distribution, virus-vector dynamics, and honeybee selective breeding. (A) contrary to previous beliefs, varroa feeds primarily on the fat body of adult honeybees and brood, which fundamentally changes our understanding of the parasite's basic biology. (B) Varroa is genetically labile, hybridizing and spilling over and back between *Apis cerana* and *A. mellifera*. (C) Varroa is also a highly efficient vector of honeybee viruses and drives changes in virus distribution, prevalence, and virulence. Despite this, some isolated bee populations survive without human intervention and (D) scientists and dedicated breeders are advancing marker-assisted selection techniques to enrich naturally occurring varroa-resistance traits in commercial stock. Bee cartoons are adapted from [78] with permission (Creative Commons Attribution 4.0 International License)

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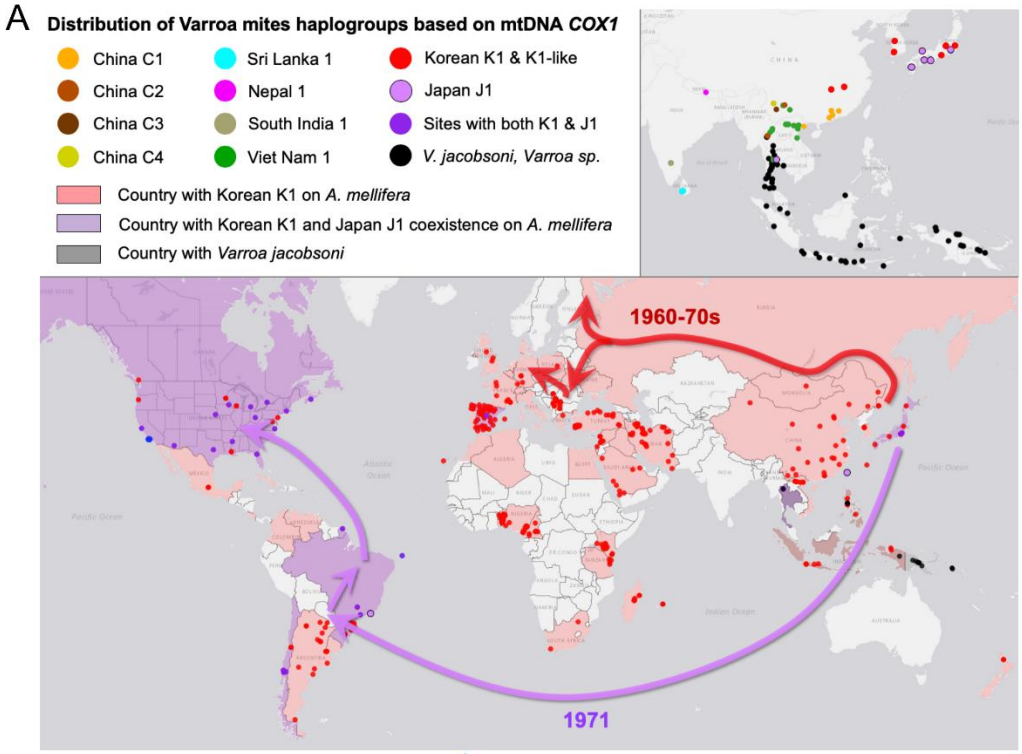

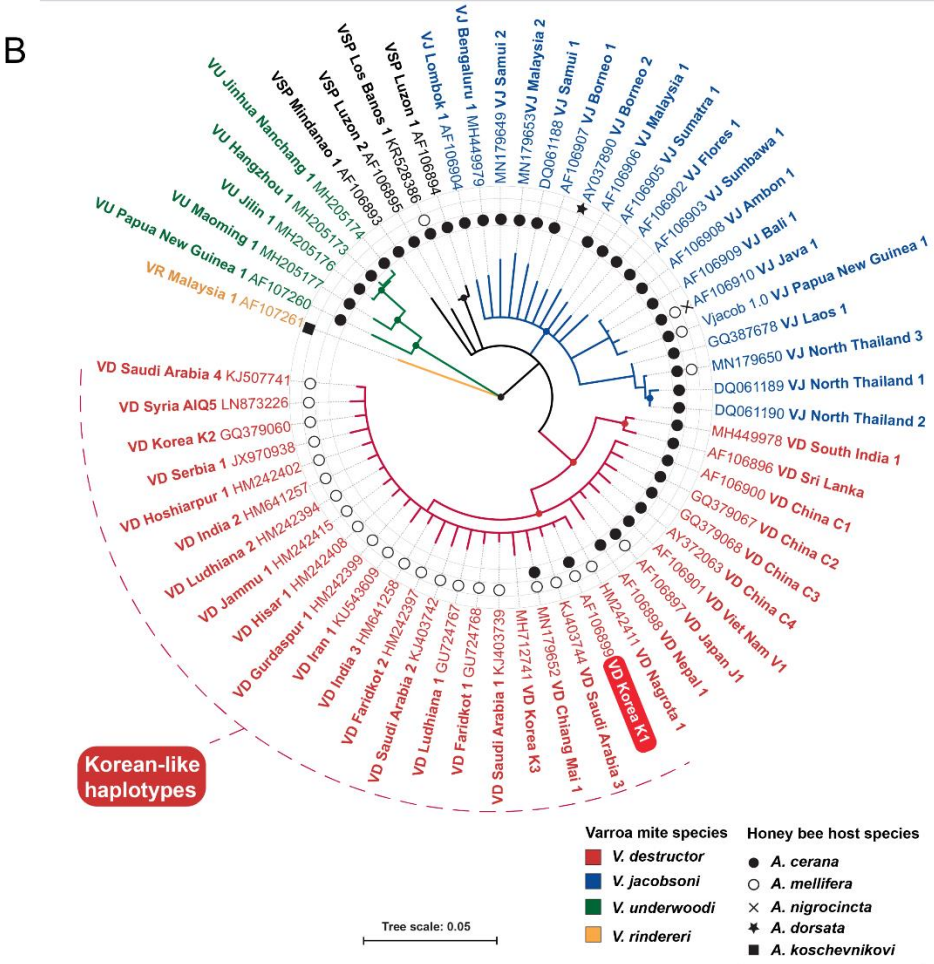

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Figure 2. Global distribution of varroa and its haplotypes. (A) Distribution of varroa haplogroups as determined by the mtDNA COX1 458 nucleotides identity on its original host *A. cerana* (inset map) and novel host *A. mellifera* (larger map). The color-coded points indicate the exact or approximated localization reported by literature and GenBank database for the Korean and Japanese strains. Arrows indicate the first known invasion waves originating from Japan and far-Eastern Russia. The number of haplotypes known for *V. destructor* differs depending on the mitochondrial markers selected (see Supplemental References) [103]. Here, 31 haplogroups were used, as reported in Supplemental Data. Interactive version of the map is available: mikheyevlab.github.io/varroa-mtDNA-world-distrib/. Constructed with Leaflet R package, ©OpenStreetMap contributors, CC-BY-SA, Tiles © Esri, DeLorne, NAVTEQ, Map tiles by Stamen Design, CCBY 3.0. (B) Phylogenetic relationship among the 60 haplogroups proposed for Varroa mites based on partial COX1 mitochondrial gene. Neighbor-joining tree using Tamura-Nei genetic distance model, *V. rindereri* as an outgroup and 1000 bootstraps. Nodes with circles indicated bootstraps over 80. Host were unspecified for 14 haplogroups in *V. destructor* mites.

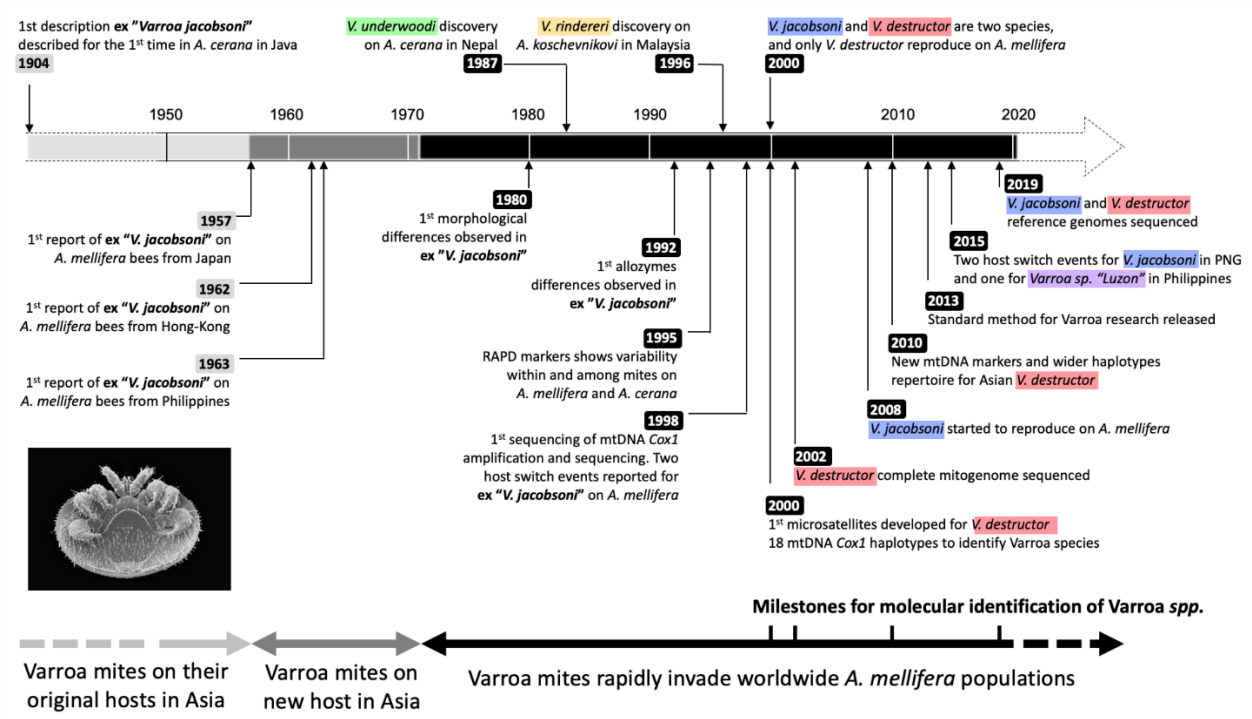

Figure 3. Timeline of discoveries. Landmarks in varroa species descriptions, global movement, and major developments in varroa research methods.

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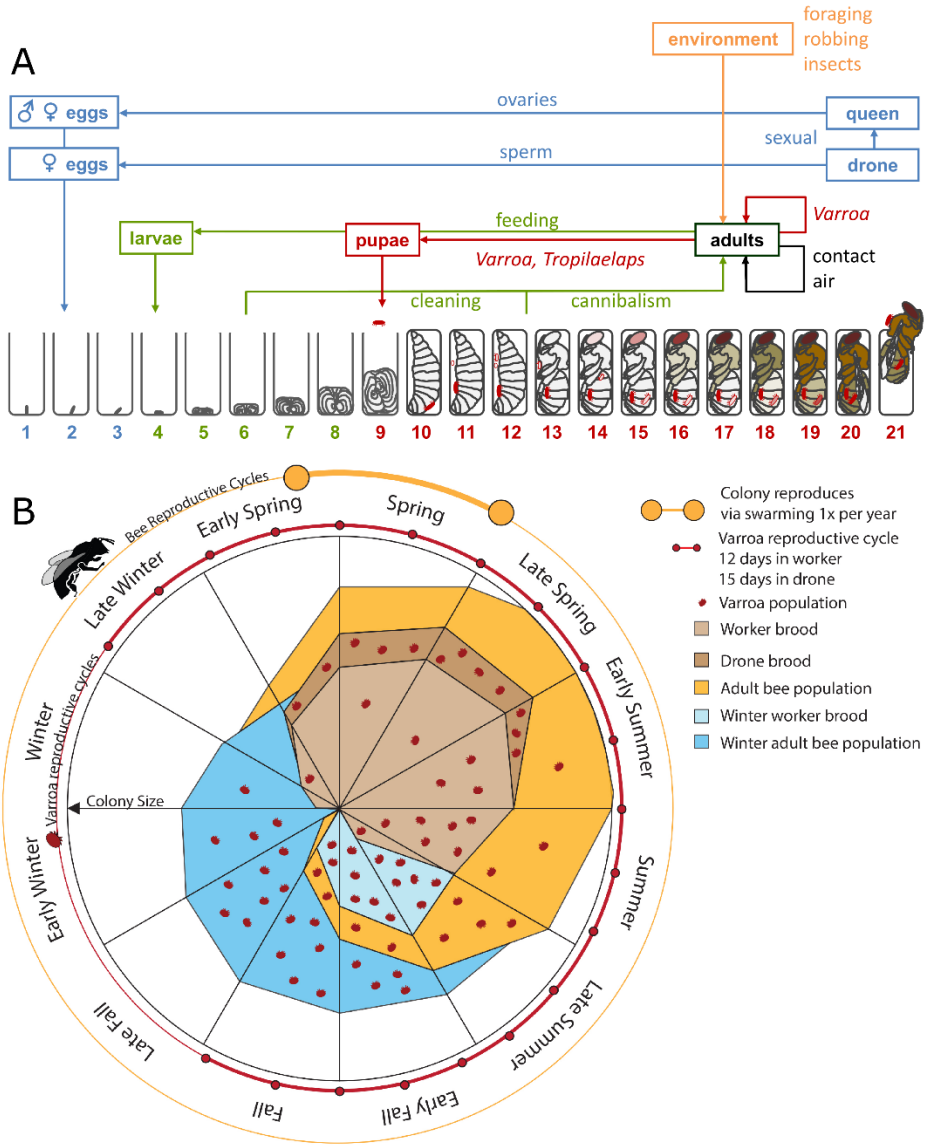

Figure 4. Varroa reproduction at the individual and colony level. (A) Individual varroa reproduction through cell invasion. While varroa transmit viruses to honeybee pupae and adults, there are many other routes of virus transmission both vertically via eggs and sperm, or horizontally via feeding, cell cleaning, cannibalism, contact, mating and ecological interactions with the environment and other insects. (B) Theoretical growth of a healthy colony without varroa mite treatments with a three-month winter. Colonies in the winter are typically comprised of all adult winter bees (dark blue) with very little worker brood (light brown) and low varroa populations. As new bees emerge, the colony expands rapidly in adult bees (yellow) and brood. By early spring the colony commences rearing drone brood (dark brown), preferentially invaded by varroa (red mites). After swarm season, bees cease rearing drones, forcing varroa to reproduce in worker brood. As mite levels increase, a single cell is co-infested by multiple foundresses, where the reproduction rate of each is reduced, but the rate of fertilized female offspring production increases. By late summer, both the bee population and brood nest area contract and varroa infestations increase above treatment thresholds on the adult bee population. Colonies simultaneously rear winter brood (light blue) that becomes the long-lived winter bees (blue) with an extra layer of fat body, which varroa feed on. As the colony stops rearing brood, varroa has no place to reproduce and their population sinks.

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Box 1: How varroa became a global parasite

Four known varroa species parasitize honeybees: *V. destructor, V. jacobsoni, V. rindereri*, and *V. underwoodi.* The first is by far the most widespread and economically damaging (**Figure 2a**). *V. destructor* switched hosts at least twice onto *A. mellifera*, probably around the 1950s. *V. jacobsoni*— also originally a parasite of *A. cerana*—independently shifted twice to *A. mellifera* in 2008 in Papua New Guinea. Its ability to spread beyond this region is not yet known. More possible jumps by undetermined species may have occurred in Philippines, but so far *V. underwoodi* remains a specialist on *A. cerana* [104] and *V. rindereri* on *A. koschevnikovi* and *A. dorsata* [105].

Until 2008, the only species parasitizing *A. mellifera* was *V. destructor* (though before 2000 it was identified as *V. jacobsoni*, until Anderson and Trueman [106] reported species differences). *V. destructor* was first reported on *A. cerana* in Java in 1904 (**Figure 3**). By 1957, it had jumped hosts to *A. mellifera* in Japan, and by 1963 in Hong Kong. Its range expanded quickly through global honeybee trade—both legal and illegal—and likely via swarms hitch-hiking on ships [107-109]. Within less than half a century, varroa spread to all regions where humans manage *A. mellifera* colonies, except Australia, some extreme northern territories, and remote islands like the Seychelles and Comoros archipelagos.

Though there are many haplogroups of *V. destructor* (**Figure 2b**), only two have successfully jumped to *A. mellifera*: the highly virulent, globally distributed Korean haplotype (K1) and the Japanese/Thailand haplotype (J1) confined to Japan, Thailand, and the Americas. K1 is thought to have first switched from *A. cerana* to *A. mellifera* near Vladivostok (north of the Korean Peninsula), while J1 made a similar jump in the 1960s following introduction of *A. mellifera* to Japan [110]. The two haplotypes are derived from genetically diverse mite populations that still infest *A. cerana* in Northeast Asia. The sympatry with *A. mellifera* offers additional spillover opportunities to other *V. destructor* lineages which cause additional threats if they spread out of Asia [103, 111]. Based on nuclear microsatellites, populations of *V. jacobsoni* and *V.*

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destructor may hybridize in *A. cerana* in Thailand, potentially indicating less host specificity and a more labile genetic population structure than previously thought [112].

Box 2: How Does Varroa Avoid Inbreeding Depression?

Intense inbreeding is common in haplodiploid systems, where the potential depression of fitness may be reduced through purging and increased purifying selection facilitated by inbreeding [113]. How varroa are able to avoid inbreeding depression (reviewed in [114]) with a reported quasi-clonality on *A. mellifera* is still a mystery. The high rate of infertility in some mature mites potentially indicates a bottleneck that selects for offspring that successfully procreate despite the necessity of mating with a brother, flushing deleterious effects from the population. Ambrosia beetles that mate with a sibling had higher egg hatching rates than outcrossed females, indicating a population that does not suffer from inbreeding depression, but is prone to outbreeding depression [115]. Similar studies have not yet been conducted in varroa, though the lack of heterozygosity despite a 10% proportion of hybrid offspring (Five F1 hybrids detected among 54 samples) suggests potential outbreeding depression, as the hybrid genetics do not enter the population [116].

Varroa engage in inbreeding most frequently during the first part of the beekeeping season, when varroa populations are low compared to host brood cells (Figure 4b). The likelihood of multiple mite invasion increases during the summer dearth, when the brood area contracts and multiple foundresses invade a single brood cell, allowing crossbreeding, recombination, and the potential spread of resistance to acaricides [117, 118]. Evidence of recombination is supported by the coexistence of diverse haplotypes and heteroplasmic mites in honeybee colonies [119].

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Outstanding Questions – How to Control Varroa Sustainably?

Biotechnical Control

While impractical on a large scale, backyard beekeepers could reduce varroa by implementing biotechnical controls and regular monitoring, including cutting drone comb, making varroa-free splits without capped brood, queen confinement to introduce an artificial brood break, colony culling, artificial swarms, and brood freezing to kill varroa. While none of these suffice on their own, inducing a brood-free stage forces varroa into the dispersal phase where they are more susceptible to organic miticides.

RNA interference and Potential Risks

RNA interference (RNAi), which enables the transient suppression of gene expression, is a budding technique for varroa control, but we should be cognizant of off-target effects. Even random sequences of dsRNA can cause shifts in gene expression, so long-term field trials must be conducted. Substantial efforts have been made to find gene targets causing mite mortality or sterility. While the finding that lithium chloride, which is commonly used in RNA purification protocols, is toxic to mites may bring previous positive results into question, the approach as a whole is not invalidated. New evidence suggests that honeybees share dsRNA between generations through jelly secretions, which may increase efficiency of dsRNA transfer between adults and brood.

Ecdysone Disruption

Mites need ecdysone hormone to stimulate vitellogenin production for their eggs, but ostensibly lack the molecular machinery to produce it themselves. Despite being necessary for insect metamorphosis, Conlon *et al.* found putative causal SNPs in varroa-resistant honeybee genes in the ecdysone-regulating pathway, making this a likely mechanism of varroa-resistance. If ecdysone-production genes can be disrupted via RNAi, varroa reproduction could be suppressed. However, it may be prohibitively difficult to ensure that this approach would not also disrupt honeybee metamorphosis.

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Glossary

American foulbrood (AFB): a fatal bacterial disease caused by the spore forming bacterium *Paenibacillus larvae*

Arrhenotokous parthenogenesis: a natural form of asexual reproduction without fertilization, where the offspring develop into males

Fat body: a critical organ in honeybees that functions like human kidneys and liver; it produces the egg-yolk precursor vitellogenin, critical for long-term survival and immune function.

Foundress: the mother mite that reproduces in a cell

Freeze-killed brood assay: a patch of brood is frozen with liquid nitrogen and the rate of removal scored. Very hygienic colonies remove the dead brood rapidly in less than 12 hours. However, this brood is simply killed through cold and so hygienic bees may not remove varroa infested cells, as varroa doesn't normally kill the brood.

Genotypic plasticity: many different genetic variations result in the same phenotype (*e.g.,* when selecting for increased hygienic behavior, different genes have been linked to the trait in different bee populations).

Grooming: a behavior where bees meticulously clean themselves or nestmates to get rid of parasites, often biting and damaging the parasite with their mandibles.

Haplogroup: for varroa, an haplogroup is an ensemble of mitochondrial haplotypes sharing identical nucleotide sequence considering the 458 bp partial fragment of *COX1* (cytochrome oxidase I subunit) but that could differ in other *loci*.

Heteroplasmy: the presence of more than one type of organellar genome in a cell or individual.

Hygienic behavior: the ability to remove dead brood rapidly from a hive, traditionally scored via the freeze-killed brood assay.

Inbreeding depression: the reduced biological fitness in a given population due to inbreeding.

Kairomone: a chemical substance (pheromone) released by one species and "overheard" by another species that uses it for personal gain, i.e. a parasite seeking a host eavesdropping on host specific chemical signals.

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Marker assisted selection: the practice of using molecular markers (DNA, RNA, or protein) as indicators for desirable phenotypes to guide selective breeding.

Dispersal phase: the adult life cycle stage of varroa, where they hitch rides and feed upon adult bee hosts.

Peritreme: in varroa, a snorkel like appendage that extends from the spiracles and allows them to breathe while submerged.

Phoretic phase: traditionally phorecy means that an organism (like a mite) uses another organism (like the honey bee) for transport, but specifically without feeding during that time. As varroa feed on their honeybee host during this phase, we advocate for a change in terminology to the **dispersal phase**.

Resistance: the ability to survive, while simultaneously reducing the agent's infectability, i.e. varroa-sensitive hygiene.

Tolerance: the ability to survive, but without reducing the parasite or viral load, i.e. bees surviving with varroa, but carrying a high viral load that can spill over to other species.

Varroa-sensitive hygiene (VSH): A form of hygienic behavior that specifically targets and removes brood infested by varroa mites.

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