Trends in Parasitology

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Review

Varroa destructor: A Complex Parasite, Crippling Honey Bees Worldwide

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The parasitic mite, *Varroa destructor*, has shaken the beekeeping and pollination industries since its spread from its native host, the Asian honey bee (*Apis cerana*), to the naïve European honey bee (*Apis mellifera*) used commercially for pollination and honey production around the globe. Varroa is the greatest threat to honey bee 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 facilitate mite dispersal and disease transmission to stronger and healthier colonies. Here, we review recent developments in the biology, pathology, and management of varroa, and integrate older knowledge that is less well known.

Highlights

Varroa destructor is an underestimated parasite: it is genetically labile, with multiple hybridization and dispersal events.

Varroa is also a highly efficient vector of honey bee viruses and drives changes in virus distribution, prevalence, and virulence.

Despite this, some *Apis mellifera* populations can survive without human intervention on all continents where varroa has dispersed.

Scientists and specialist breeders are advancing marker-assisted selection techniques to enrich naturally occurring varroa-resistance traits in commercial stock – an approach predicted to be most effective when combined with culling susceptible colonies.

Culling is identified as an effective means of removing both undesirable genetics and varroa-related virulence.

A Formidable Foe

Remarkably adaptive and complex (Figure 1, Key Figure), *Varroa destructor* [1] (hereafter referred to as varroa, unless otherwise stated) is linked to the worldwide decline in honey bee (*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** (see Glossary) have been defined (see Boxes S1–S3 in the supplemental information online), the Korean K1 is the most pervasive (Figure 2B). No other pathogen or parasite has had a comparable impact on honey bees, in part because varroa only recently adapted from its original host, the Asian honey bee (*Apis 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, entombment of drone brood infested with multiple mites, and increased **hygienic behavior** (reviewed in [4]). The recently updated varroa genome (GCA_002443255.1) [5] will be a powerful tool to help understand varroa evolution in response to novel honey bee defense traits defense traits, host-switching, and successful global invasion.

Varroa mites are 'wingless, eyeless, and unable to crawl between widely spaced honey bee nests' [6]. Yet monitoring efforts show that honey bee colonies are almost universally infested [2]. Colonies often experience unnatural surges of varroa when nearby colonies collapse [7], potentially due to drift, and definitely due to robbing, when bees from healthy colonies exploit poorly defended, collapsing colonies to steal honey [6]. These varroa-laden, collapsing colonies complicate varroa control. Furthermore, varroa is a dangerously efficient vector of several bee viruses, which has dramatically worsened the virus landscape [8]. We have underestimated varroa's adaptive ability: the mite has expanded its host range multiple times (Box 1), has excellent chemosensing abilities [9], engages in chemical mimicry [10], and manipulation of its host [11], readily disperses within and between colonies [6,11], engages in parental care [12], and rapidly evolves acaricide **resistance** [13]. Apicultural practices create a virtually limitless supply of new host colonies. Most colonies are treated prophylactically with acaricides, limiting

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Key Figure

Advances in Our Understanding of *Varroa destructor*

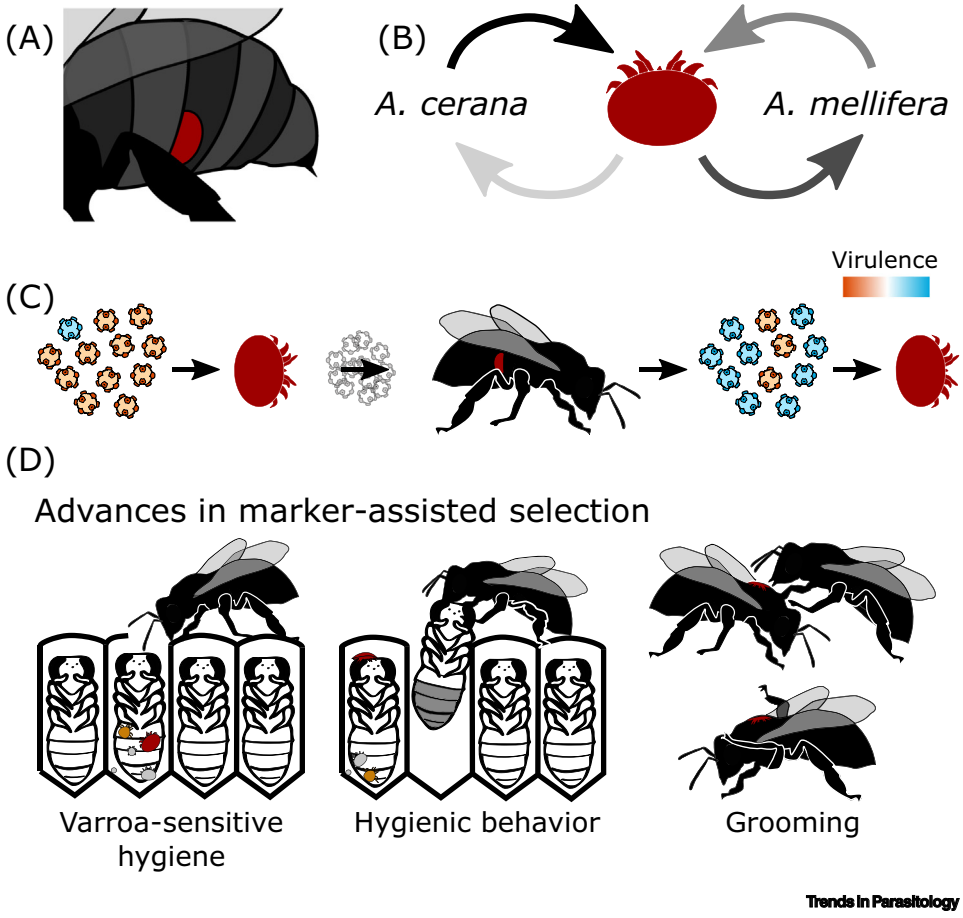


Figure 1. In this review we examine varroa's biology, distribution, virus–vector dynamics, and honey bee selective breeding. (A) Contrary to previous beliefs, varroa feeds primarily on the fat body of adult honey bees and brood, which fundamentally changes our understanding of the parasite's basic biology. (B) Varroa is genetically labile, hybridizing and spilling over and back between *Apis cerana* and *Apis mellifera*. (C) Varroa is also a highly efficient vector of honey bee viruses and drives changes in virus distribution, prevalence, and virulence. Despite this, some isolated bee populations survive without human intervention. (D) Scientists and dedicated breeders are advancing marker-assisted selection techniques to enrich naturally occurring varroa resistance traits in commercial stock.

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 honey bee brood cells, where a **foundress** mite raises her young
- The **dispersal phase** – often incorrectly termed the **phoretic phase** – in which mature female mites travel and feed on adult bees

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.
Dispersal phase: the adult life cycle stage of varroa, where they hitch rides and feed upon adult bee hosts.
Fat body: a critical organ in honey bees 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 h. However, this brood is simply killed through cold and so hygienic bees may not remove varroa-infested cells, as varroa does not 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 in which bees meticulously clean themselves or nestmates to get rid of parasites, often biting and damaging the parasite with their mandibles.
Haplogroup: a haplogroup is a collection of organisms (varroa mites in this case) with identical haploid genotypes. The haploid genotypes are commonly defined by the nucleotide sequence of a small representative fragment of either a nuclear gene (locus) or a mitochondrial gene (usually the cytochrome oxidase I subunit, or COX1). Individuals can therefore belong to different haplogroups, depending on which locus is investigated.
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, that is, a

Box 1. How Varroa Became a Global Parasite

The first report of varroa was on *A. cerana* in Java in 1904 by Oudemans (Figure 3). Four known varroa species parasitize honey bees: *V. destructor, V. jacobsoni, Varroa rindereri*, and *Varroa 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 Papua New Guinea. Its ability to spread beyond this region is not yet known. More possible jumps by undetermined species may have occurred in the Philippines, but so far *V. underwoodi* remains a specialist on *A. cerana* [95] and *V. rindereri* on *Apis koschevnikovi* and *A. dorsata* [96].

Until 2008, the only species parasitizing *A. mellifera* was *V. destructor*, though before 2000 it was identified as *V. jacobsoni*, until Anderson and Trueman reported species differences (Box S1). By 1957, it had jumped hosts to *A. mellifera* in Japan, and by 1963 in Hong Kong. Its range expanded quickly through global honey bee trade – both legal and illegal – and likely via swarms hitch-hiking on ships. 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 such as the Seychelles and Comoros archipelagoes (Figures 2 and 3).

Though there are many haplogroups of *V. destructor* (Figure 2B, Box S2), 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 (Box S3). 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 late 1950s following introduction of *A. mellifera* to Japan. The two haplotypes, which can hybridize, are derived from genetically diverse mite populations that still infest *A. cerana* in Asia. While mtDNA indicated that Japanese mites may be displaced by Korean mites, the extent of J1 genetic contribution to invasive varroa populations via admixture remains poorly understood [97]. The sympatry with *A. mellifera* offers additional spillover opportunities to other *V. destructor* lineages which cause additional threats if they spread out of Asia. Based on nuclear microsatellites, populations of *V. jacobsoni* and *V. destructor* may hybridize in *A. cerana* in Thailand, potentially indicating less host specificity and a more labile genetic population structure than previously thought [98].

parasite seeking a host eavesdropping on host-specific chemical signals.

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

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

Phoretic phase: traditionally, phoresy means that an organism (such as a mite) uses another organism (such as the honey bee) for transport, but specifically without feeding during that time. As varroa feeds on the honey bee 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, that is, varroa-sensitive hygiene.

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

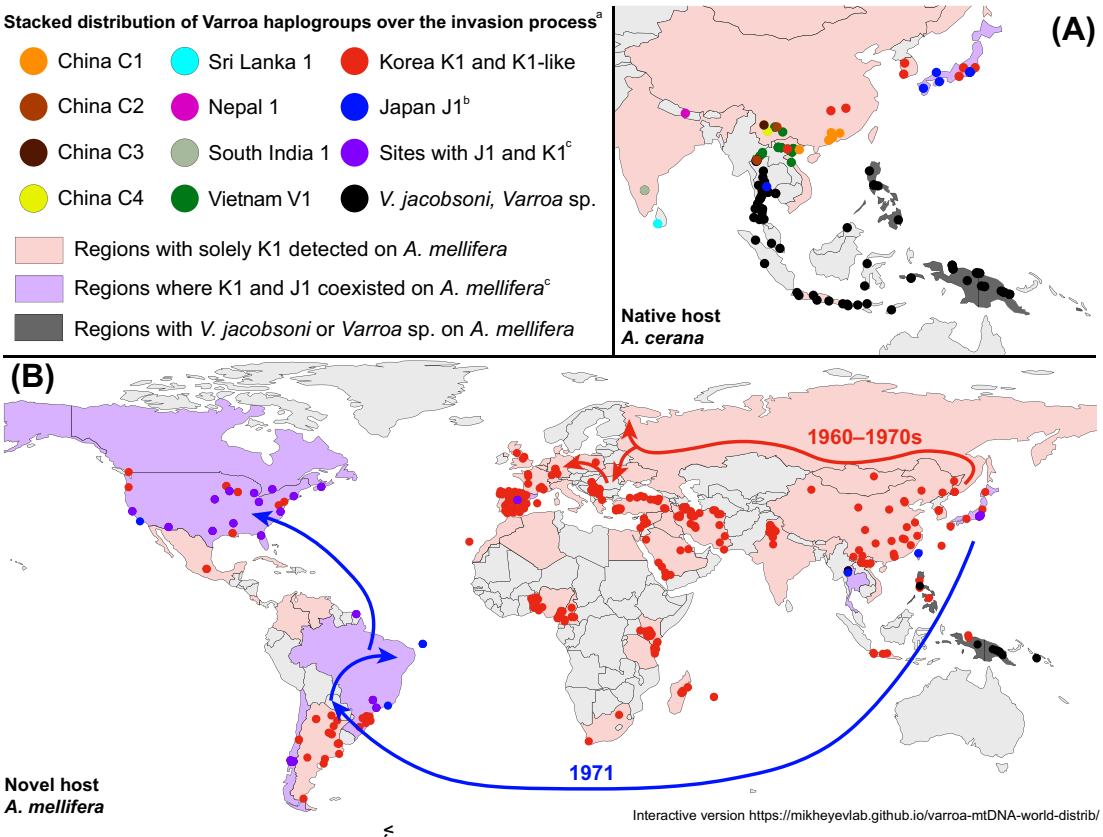
Reproductive Phase

In *A. mellifera*, varroa typically produces 0.7–1.45 mature female daughters in worker brood cells and 1.6–2.5 daughters in drone cells (reviewed in [1]). Varroa mites use **kairomones**, a form of 'chemical espionage' to invade appropriately aged larval cells [14] (Figure 4A), exhibiting an eight-fold preference for drone brood, where they have increased reproductive potential [15]. The amount of available worker and drone brood changes throughout the season, impacting the proportion of varroa in brood versus on adult bees (Figure 4B). Upon invading the brood cell, the foundress hides, immobile, in the pool of food at the base of the cell, breathing through her **peritreme** that extends above the liquid food like a straw [1]. This immobility may be an adaptation to minimize removal by worker bees, as, prior to and during capping, nurse bees frequently inspect the cell. After cell capping, the honey bee larva finishes the brood food, stretches out along the length of the cell and spins a cocoon. During this stage, the mite leaves the brood food, climbs onto the bee prepupae, and punctures a relatively large hole (100 μm) in the bee's cuticle to create a feeding site for herself and future offspring [16]. This feeding site remains open due to anticoagulants in mite saliva and suppression of host wound healing [17].

Feeding on the larval **fat body** is a prerequisite for varroa reproduction [18]. Signals from the bee larva trigger mite reproduction [19] and influence the gender of the egg [20]. Sex determination in varroa is via **arrhenotokous parthenogenesis**: males are haploid with seven chromosomes, while females are diploid with 14 chromosomes. However, the exact genetic mechanism (e.g., the existence and identity of a distinct genetic sex-determination locus) remains unknown.

Initiating Oviposition

Initiating oviposition is an energetically demanding task, and foundresses derive this energy by metabolizing consumed honey bee tissue. Proteomic [21] and transcriptomic [22] studies identified a drop in carbohydrate metabolic enzymes during foundress egg-laying, whereas they are upregulated before and after egg-laying [22]. This expression pattern matches remarkably well



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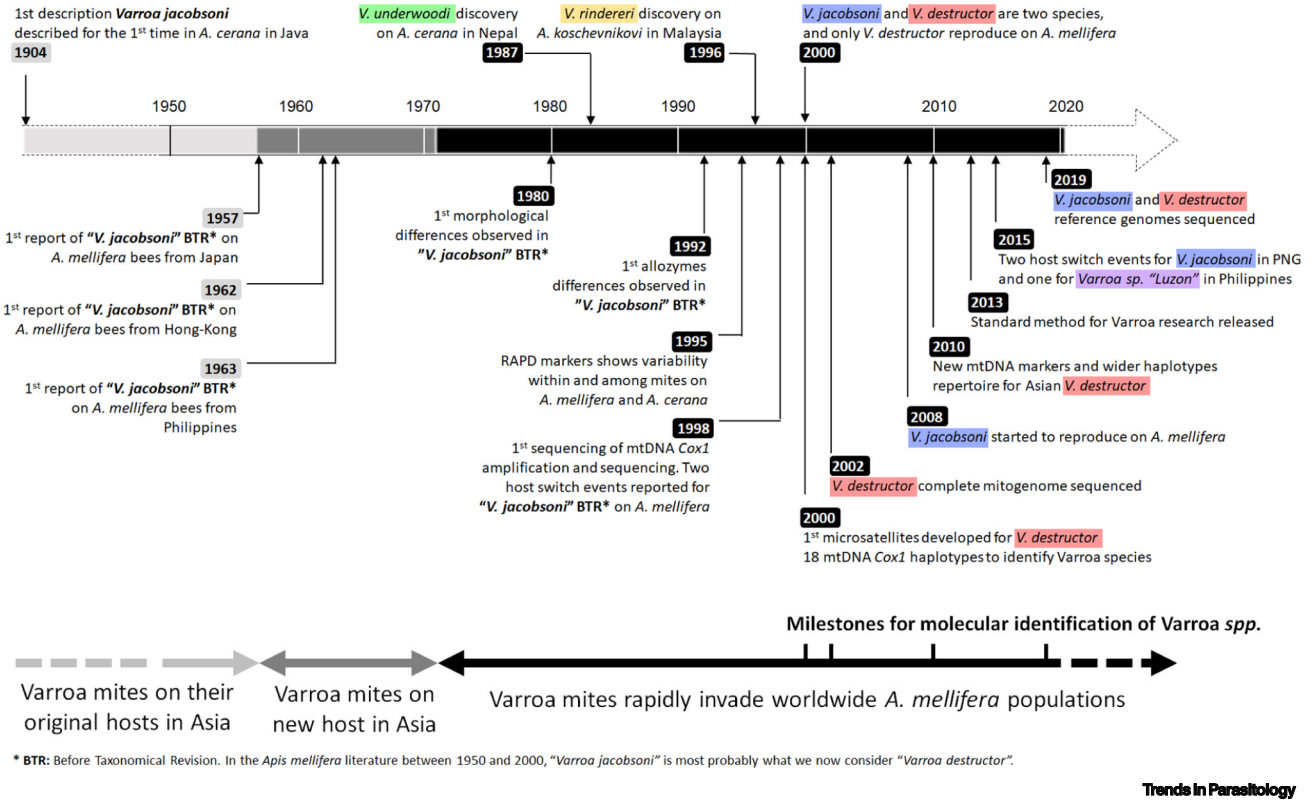

Figure 3. Timeline of Discoveries. Landmarks in varroa species descriptions, global movement, and major developments in varroa research methods. See Table S1 (in the supplemental information online) for a list of supporting references in chronological order. Abbreviations: PNG, Papua New Guinea; RAPD, random amplification of polymorphic DNA.

with periods of unmetabolized nutrient transfer from the host [23] and the foundress [24] 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 [23,25]. 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 and engages in careful parental care, gluing the egg to the upper cell wall (the safest spot during

Figure 2. Global Distribution of Varroa and Haplotype Co-occurrence. Time stacking of varroa haplogroup distribution, determined by mtDNA COX1 458 nucleotide identity on (A) *Apis cerana* and (B) *Apis mellifera*. Colored points indicate the location reported by literature and GenBank database for all haplogroups on *A. cerana* and the *Varroa destructor* Korean and Japanese strains on *A. mellifera*. Arrows indicate invasion waves from Japan and far-Eastern Russia following host switching. Thirty-one haplogroups were used. See Boxes S1–S3 for a complete discussion on the methods we used. Interactive maps with details of origins and time acquisition are available on github.com/MikheyevLab/varroa-mtDNA-world-distrib/ and public domain map Natural Earth Vector from github.com/nvkelso/natural-earth-vector/. [a]Some haplotypes may have been eradicated by human intervention or may have never been reported after the first introduction. [b]Reports of J1 mites on *Apis mellifera* samples collected in Japan between 1989 and 2015 used a variety of techniques from random amplification of polymorphic DNA (RAPD), PCR-restriction fragment length polymorphism (RFLP), to Sanger sequencing. [c]J1 and K1 mites coexisted for either a short or longer period of time with the possibility of hybridization. Although these same locations may currently be dominated entirely by K1 origin mites according to mtDNA markers, the extent of admixture is not known in most regions. (C) Phylogenetic relationships among the 60 haplogroups proposed for varroa mites based on the partial COX1 mitochondrial gene. Neighbor-joining tree using Tamura-Nei genetic distance model, *Varroa rindereri* as an outgroup, and 1000 bootstraps. Nodes with circles indicate bootstraps over 80. The host was unspecified for 14 haplogroups in *V. destructor* mites.

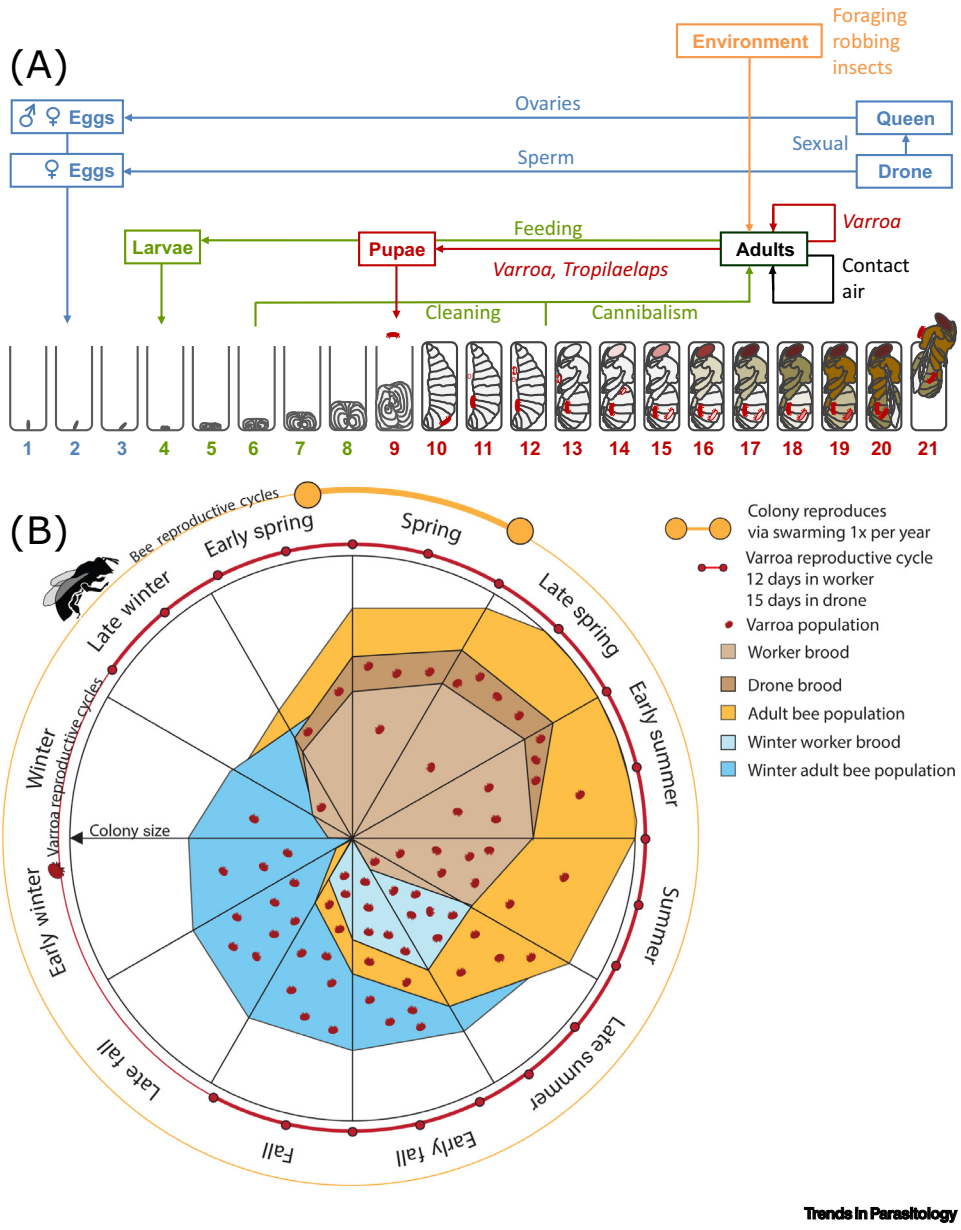

Figure 4. Varroa Reproduction at the Individual and Colony Level.

For a Figure360 author presentation of Figure 4B, see the figure online at http://dx.doi.org/10.1016/j.pt.2020.04.004#mmc2. (A) Individual varroa reproduction through cell invasion. While varroa transmits viruses to honey bee pupae and adults (red arrows), there are many other routes of virus transmission, such as sexual and vertical transmission via eggs and sperm (blue arrows), or horizontal oral and fecal transmission via feeding, cell cleaning, cannibalism (green arrows) contact transmission between adults (black arrows), and ecological interactions with the environment and other insects (orange arrows). (B) Theoretical growth of a healthy colony without varroa mite treatments with a 3-month winter. Colonies in the winter typically consist of 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 coinfested by multiple foundresses where the reproduction rate of each is reduced, but the rate of production of fertilized female offspring 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.

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 [99]. How varroa is able to avoid **inbreeding depression** with a reported quasiclonality 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. Interestingly, 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 [97].

Varroa engages in inbreeding most frequently during the first part of the beekeeping season, when varroa populations are low compared with 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 cross-breeding, recombination, and the potential spread of resistance to acaricides [100].

bee pupation) to ensure that the male protonymph can walk away after hatching [12]. Male mites often die during host pupation, due to pupal movement and the pupal legs blocking his access to the communal feeding site [26]. The foundress then lays a diploid egg every 30 h thereafter, depositing them further down the cell wall. When the first female matures, she mates with her brother (Box 2) on the communal fecal pile. Males mate almost exclusively with freshly molted females, and stop mating with older females when a younger one finishes her last adult molt [27]. If the male is dislodged or dies, the females emerge unmated. Formerly, it was believed that these unfertilized varroa were unable to mate once they left 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 [28]. Mites do not always initiate oviposition after cell invasion [29], potentially due to disruption in chemical communication. Indeed, oviposition can be experimentally disrupted by applying (Z)-8-heptadecene to brood cells before capping [30], and other compounds can disrupt host-seeking behavior [31].

Under laboratory conditions, a female mite can have up to seven reproductive cycles during her lifetime and lay up to 30 eggs [32]. 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 [33]. In an *A. mellifera* worker brood, a foundress with seven reproductive cycles would theoretically produce ~ 5–10 mature daughters, or ~ 10–17 mature daughters in drone brood. However, under field conditions each mite has only ~ 1.5 to 3 reproductive cycles [1].

Dispersal Phase

When a parasitized honey bee emerges from its cell, it carries the mature female mites (mother and daughters). The daughters frequently switch to a nurse-aged bee [11] to activate their ovaries, allow the spermatophores to mature [33], 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 [34]. Varroa waste consists predominantly of guanine with traces of hypoxanthine, uric acid, and caffeine [35], suggesting a protein-rich diet with limited water. New research has overturned the decades-long belief that varroa feeds exclusively on hemolymph, demonstrating that feeding on the fat body is required for varroa egg production [18] and that fat-body tissue was consistently detected inside the gut of mites feeding on honey bee adults [36]. Inspection of varroa feeding sites revealed feeding holes between the overlapping abdominal plates of the honey bee and degraded fat body cells beneath the intersegmental membrane, likely due to extraoral digestion by mite saliva [36].

Parasite-Induced Changes Enable Colony Dispersal

Varroa mites alter the hydrocarbon cuticle of their hosts and adapt their preference for different adult-host life stages based on colony infestation levels [37]. At low mite abundance, varroa preferentially parasitizes nurse bees, which frequently tend to brood and thus provide opportunities to infest an appropriately aged larval cell. Varroa distinguishes nurse bees from foragers by different chemical cuticular signatures [38]. When mite abundance increases in the colony, the chemical profile of nurses and foragers tends to overlap, promoting mite departure by dispersing onto foragers [37]. Parasitized brood develops into adult bees that spend less time nursing, mature at an accelerated rate [39], contribute less to colony productivity, and potentially promote varroa dispersal to new colonies [1,40].

Virus Transmission

By feeding on bee tissues, varroa acts as an efficient vector of pathogens. 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 the pathogen into the new host

The efficacy of vector-mediated virus transmission depends on secondary conditions, such as what pathogens are present where the mite is feeding, pathogen survival between 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) [41,42]. These conditions differ significantly between individual viruses, impacting their virulence and their relationship with varroa [40].

Viruses Associated with Varroa

Many viruses have been detected in honey bees, with new potential viruses being discovered constantly [43]. Many honey bee viruses can be efficiently propagated by injecting them into pupae or adult bees [41,44,45], similar to how varroa feeds on its host. In theory, therefore, all of these viruses can be transmitted by varroa. However, in practice only Deformed wing virus (DWV) and Acute bee paralysis virus (ABPV) have a clear varroa–vector relationship [46,47]. Both DWV and ABPV have several major co-circulating variants that differ in virulence characteristics [41,42,46,48–51]. Sacbrood virus (SBV) does not seem to be transmitted directly by varroa but is a co-factor in natural varroa resistance/survival and virus adaptation [52], DWV-induced bee mortality [45], general virus–host interactions and immunity [53], intervirus competition [48], and varroa behavior [54]. SBV induces pollen aversion in bees and has therefore a strong effect on nursing, division of labor, foraging, and bee nutritional status [44], which themselves play major roles in varroa-virus virulence [39]. For other viruses, the relationship with varroa is indirect or nonexistent [55,56], the most extreme example being Slow bee paralysis virus (SBPV), which can be transmitted by varroa both individually and epidemically [57], but whose natural prevalence in honey bee colonies remains marginal [56].

Many bee viruses use several modes of transmission, each with its own virulence rules and needs, allowing different virus variants to coexist simultaneously [41,42,45,49,50,58]. Host range and geographic isolation are two other common sources of major virus variants [47], 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. Recombinant viruses, where variants exchange whole genome sections, can be particularly virulent [49] because they combine the strongest parts of the variants into a single genome.

The co-persistence of these variants in the bee population depends on the costs and benefits of virulence, for virus and host. The cost of DWV-B's higher individual-level virulence, relative to DWV-A, in both varroa [42,59] and honey bees [41], is greater neurological impairment of its bee [41] or mite [54,59] host, which can be either beneficial [51] or detrimental [60] at the social level, depending on colony developmental stage, the dominant local DWV strain [51], and especially the contrasting effects of virulence on social hygienic behavior [61–64].

Virus Symptoms and Their Significance

Apart from the secondary factors mentioned above, colony mortality (particularly, its timing during the bee season) strongly influences the real-world relationship between varroa and individual viruses [40,65]. As varroa is an obligate parasite, its survival is intimately coupled to the availability of colonies, which beekeeping readily supplies. 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) 'reinvasion' into a strong colony immediately prior to overwintering [1]. The colony-level symptoms of DWV in autumn are therefore important features of varroa survival and transmission between colonies [40]. The colony-level symptoms and mortality associated with varroa-vectored ABPV infections occur earlier in the summer [65], prior to the robbing season, hence missing the opportunity to transfer varroa into colonies capable of overwintering [40].

Viral infection can also impair honey bees' social immunity defenses. For example, DWV-infected honey bees are less able to differentiate between varroa-infested and noninfested pupae [64]. Moreover, ABPV-infected pupae are efficiently removed by **varroa-sensitive hygienic (VSH)** behavior (Figure 1D), while DWV-infected pupae are not [61], which favors varroa-mediated transmission of DWV over ABPV. Similarly, hygienic bees preferentially identify [41] and remove [62] pupae infected with the more virulent DWV-B variant, thus helping the less virulent DWV-A variant to 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 [55]. Overall, varroa is both vector and host for certain bee viruses, particularly DWV-B, and is also behaviorally affected by both DWV and SBV [54], adding a whole new range of factors to the dynamic between varroa, bees, and viruses.

Virus Ripple Effects in New Territories

The 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 [66] and spread into all regions of the country by 2013. The initial arrival of varroa led directly to a 16% drop in colony numbers [67]. This wave of varroa expansion provided a unique opportunity to compare virus expression between parasitized and nonparasitized colonies [55]. The rapid spread of varroa across the mainland of the country – less than 15 years to cover a 1600-km territory – was accompanied by a dramatic change in the viral landscape, with each virus responding in a unique way [55].

Varroa's clear role in spreading the more virulent strains of different viruses was repeated in the Hawaiian archipelago [46]. The dynamic shifts in the observed viral titers suggest that the multiple viruses in honey bees interact to create a changing pathological landscape that peaks soon after varroa arrival [2–3 years for Kashmir bee virus (KBV), SBV, and black queen cell virus (BQCV)], before becoming more stable and predictable depending on the level of varroa infestation [55,68]. 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 [55].

Viruses, Varroa Thresholds, and Virulence Management

If virulence is not punished, it will proliferate. Keeping weak colonies alive during winter, through intensive varroa management or by combining with strong colonies, encourages the transmission and survival of virulent varroa and virus traits, much like reinvasion [40]. One of the most important, and least adopted, practices in virulence management is culling, which is largely absent in beekeeping other than for **American foulbrood (AFB)**. Since the only host for varroa is the honey bee, which is overwhelmingly controlled by beekeepers, culling would be particularly effective for removing inadequate honey bee genetics and virulent varroa-virus traits [40] (see Box S4 for further discussion on culling).

Social Immunity

In comparison with 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 [69]. This 'social apoptosis' largely limits successful varroa reproduction to drone larvae [69,70], which disrupts the mite's reproductive cycle and may produce a stronger stimulus for hygienic behavior through larval decay [70]. Varroa toxic protein does not have the same lethality on *A. mellifera* larvae, thereby increasing the amount of suitable brood to support mite proliferation [69]. New research shows that brood-related traits in *A. mellifera* could contribute to hygienic behavior, VSH, and the suppression of mite reproduction [71]. Brood frames transplanted from nonhygienic to hygienic hives and vice versa produced hygienic scores correlated with the donor colony, rather than the recipient colony [25,61,71]. 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

Allogrooming and autogrooming contribute to varroa resistance by removing mites from adult bees and also by physically damaging the mites, preventing them from seeking a new brood cell to infest [72]. Honey bees can initiate allogrooming via a 'grooming invitation signal' – a whole-body vibrational dance lasting several seconds – which stimulates other workers to groom the dancer. Grooming workers use their mandibles and forelegs to forcefully remove the mites from adult bees, leading to mite injury or death [73]. In the USA, scientists have produced a strain of bees, now commercially available, that exhibit elevated grooming and mite biting [74].

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 damaged 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 [61,75,76], but contrary to conventional wisdom, all of these molecules are relatively nonvolatile. We speculate that either hygienic workers are extremely sensitive to these compounds, or workers may periodically open and inspect brood cells [77,78], looking for stronger contact cues.

Hygienic removal of varroa-infested cells (also called VSH) is a specific subcategory of hygienic behavior that is only partly predicted by tests such as the **freeze-killed brood assay** [79]. VSH-specific brood effects reduce mite fecundity [61,76], and high-VSH colonies preferentially remove brood infested with multiple foundresses [63] or foundresses carrying highly virulent

viruses [41,61,62]. Removing multiply infested cells also deters varroa outcrossing, potentially inhibiting the spread of genetic traits like acaricide resistance (Box 2). Likewise, preferential removal of varroa carrying highly virulent virus 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 many differential expression studies analyzing transcript and protein profiles associated with hygienic behavior, VSH, and to a lesser extent grooming (also reviewed in [80]). 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 [80]. There is a disconcertingly low degree of congruency among the differentially regulated genes identified by different researchers. However, most have generally concluded that olfaction, neural signal transduction, and ligand degradation are key molecular processes underlying hygienic behavior and VSH [80]. 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**, that is, different biochemical pathways (with presumably different underlying genetic control) ultimately result in similar phenotypes. However, these are mostly behavioral traits, and are thus also subject to colony-level dynamics and their internal and environmental drivers, which could be further sources of poor congruency.

Honey Bee Populations Surviving with Varroa

When varroa first arrives in 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 [81].

Developing Varroa Resistance without Human Intervention

Evidence of adaptation can be seen in several subspecies, including *Apis mellifera scutellata*, *Apis mellifera capensis*, and multiple populations of Western honey bees (ssp. *carnica*, *mellifera*, and hybrids) (reviewed in [81]). In contrast to active selection via controlled breeding programs, natural selection gears towards host–parasite equilibrium within the context of the local environment. Populations of surviving bees within Europe and North America were likely founded by unmanaged feral colonies, 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 with sympatric control colonies [81].

Conserved Traits in Naturally Resistant Bees

Varroa-resistant populations share traits that permit survival despite parasite infection (reviewed in [80,81]). One of these adaptations is an elevated rate of recapping behavior, which may disrupt varroa reproduction without social apoptosis, thereby reducing the colony-level cost of natural varroa defense [77]. The Gotland 'Bond bees' and the Arnot forest bees have smaller colony sizes than commercial stocks and a greater tendency for swarming [82,83], whereas the Gotland and French populations both display reduced varroa reproductive success [84]. Evidence for VSH and grooming in naturally adapted populations has so far been mixed [85–87]. Despite adapting independently, the surviving populations seem to share a handful of traits that work additively and permit survival [77,81]. However, these traits are often misaligned with commercial needs, where large populations, early and prolonged brood rearing, and no swarming are prized.

Breeding Commercially Viable Resistant Stock

Some commercial beekeepers have stopped chemical varroa interventions and continue to select for commercially desirable traits such as honey production in bees in France [88] and Norway [86]. These populations display the same reduced mite reproductive success seen

elsewhere [77], potentially due to ecdysone disruption in the brood [25] (which varroa requires for reproduction but cannot biosynthesize) or due to interruptions in the reproductive cycle by behaviors such as brood removal and cell recapping. Some scientists have called for new breeding methods that do not involve regular acaricide treatment, advocating that, by increasing selective pressure, natural selection will achieve host–parasite equilibrium [89]. However, because of horizontal parasite transfer [6] this could threaten the livelihood of surrounding beekeepers.

An integrative method, involving treatment by necessity, selective queen rearing, and culling of highly infested colonies, is recommended for those attempting to breed varroa-resistant bees. While field assays for measuring resistance traits, like the VSH and grooming tests, are prohibitive for large-scale beekeeping operations, **marker-assisted selection** via genetic or proteomic testing has been demonstrated to be economically viable [90]. However, if the speculated genotypic plasticity described above is occurring, that would mean that different honey bee populations may have different genetic or expression markers, which would complicate the utility of this approach. This hypothesis remains to be tested and will be an important step for determining the usefulness of this technique on a large geographic scale.

Concluding Remarks

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 [91]. Additional insight into varroa's basic biology, genetic architecture, and demographic history are necessary to develop sustainable control measures and breeding programs. The updated varroa genome [5] is a step toward leveraging population genomics and understanding varroa diaspora success despite limited genetic diversity. However, we should simultaneously prepare for two other mites that may soon spread worldwide. The cryptic *Varroa 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 host species, *Tropilaelaps* spp., has shown similar adaptive shifts, from its original host, the Giant honey bee (*Apis dorsata*) to *A. mellifera*. Currently, only two of four species (*Tropilaelaps mercedesae* and *Tropilaelaps clarae*) parasitize *A. mellifera* [92], with *T. mercedesae* exhibiting a wider geographical distribution, which is still limited to East Asia. 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 use against *Tropilaelaps*. Nevertheless, urgent research on how this pest has adapted to its new host is critical.

In Southeast Asia, co-infestation of *V. destructor* and *T. mercedesae* is common, and *Tropilaelaps* mites often outcompete varroa [93]. 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 [94]. Experience with varroa and its rapid spread (Figure 2, Box 1) suggests that range expansion of *Tropilaelaps* is only a matter of time, and countries should prepare for its arrival.

Outstanding Questions

How can beekeepers control varroa sustainably at a commercial scale?

Will RNAi, a technology that suppresses gene expression, become a feasible approach for varroa control?

Will marker-assisted selective breeding techniques for varroa resistance be economically feasible for large-scale queen breeders?

Will beekeepers be prepared for the arrival of novel parasites?

How do we balance individual livelihoods of beekeepers with the best collective response to invasive parasites?

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