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Genetics and physiology of *Varroa* mites

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Varroa destructor is the primary biological threat to domesticated honey bee colonies in much of the world, impacting host fitness both directly and by transmitting RNA viruses. Genomic, proteomic, and functional-genetic resources provide a framework for *Varroa* biology. When coupled with physiological analyses of development, host finding, and reproduction, these resources reveal general traits of arthropods and offer new strategies for mite control. Efforts to develop novel controls are focused on efficacy, efficient delivery, and the avoidance of both host impacts and the swift evolution of resistance by mites.

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Introduction

Varroa destructor is the most important parasite of the ubiquitous European honey bee, *Apis mellifera*. This mite causes honey bee colony losses and increased management costs in the countries to which it has spread, and substantial surveillance costs in countries fortunate enough to have escaped its presence thus far. *V. destructor* originated in Asia after shifting hosts from the Asian honey bee *Apis cerana* to introduced populations of *A. mellifera*. With this host shift came the ability to parasitize female worker-destined larvae in *A. mellifera* colonies, as well as male-destined (drone) larvae. *Varroa* species that parasitize *A. cerana* are confined to seasonal and less numerous drone larvae, decreasing growth potential and arguably impacting a caste that is less critical for colony growth and disease transmission. Determining how the host cues perceived by Varroa changed with respect to this speciation event remains a key goal in understanding and perhaps controlling these mites. *V. destructor* benefits from the nearly worldwide distribution of *A. mellifera* coupled with management practices, including unnaturally high colony densities that favor horizontal transmission of disease across colonies [1]. *V. destructor* is one of the most successful of all mite species, crippling honey bee colonies in Africa, Asia, Europe, the Americas, and much of Oceania.

Genetic tools can shed light on the dispersal and mating of this parasite, its abilities to vector viruses to bee hosts, host recognition, and the unique physiology that has allowed it to survive defenses by both bees and beekeepers as it thrives in *A. mellifera*. This review will highlight emerging genetic resources and insights into *Varroa* biology, coupled with advances in discovering and exploiting weaknesses of this highly specialized mite.

Population genetics and the *Varroa* diaspora

Varroa appears to have successfully leapt from *A. cerana* to *A. mellifera* at least twice, leading to lineages referred to as the 'Korean' and 'Japan' strains [2]. Of these two widespread *V. destructor* strains, the Korean strain prevails, having spread throughout Asia and Europe and soon thereafter into the Americas, eventually reaching back across the Pacific Ocean to New Zealand [3•]. The Japan strain is found in parts of this same range but at low frequencies, suggesting lower reproductive success by mites of this strain. The extent of admixture between these strains remains murky, and is a key objective of population-genomic approaches for *V. destructor*. Unfortunately, recent experimental and genetic research indicates that *Varroa* species currently parasitizing *A. cerana* continue to test *A. mellifera* as a new host, with at least some success [4•,5].

V. destructor shows the reduced genetic diversity expected for a rapidly expanding invasive species [3•] although recent estimates show that nucleotide diversity is non-trivial and perhaps increasing despite a mating system that favors inbreeding [6]. Key research gaps remain for *Varroa* population genetics, including the resolution of gene flow patterns across and between continents, determining mite sources for recent island invasions, and assessing mite movement between colonies and apiaries.

Genomic and proteomic resources

Thanks to its economic impacts, *V. destructor* has been the focus of genomic and transcriptomic sequencing efforts, leading to a published survey sequence [7], a 'version 2.0' assembly and gene set (https://www.ncbi.nlm.nih.gov/assembly/GCA_000181155.2), and a 'version 3.0' assembly that will lead to a reference genome and gene set

Box 1 Are we breeding meaner mites?

Host-parasite evolution suggests at least two traits common to beekeeping will favor the evolution of greedier parasites with more severe impacts on their hosts (virulence): firstly, High colony densities and movement that allow parasites to leave failing colonies and exploit new ones and secondly, control methods that knock parasites back repeatedly to a small founding population. Several parts of the world show signs of equilibrium between bees and *Varroa* mites, with honey bee colonies resisting or tolerating mites despite minimal beekeeper inputs. Genetic screens can now be used alongside life history analyses to determine whether the mites from populations under different management schemes differ in surrogates for virulence such as reproductive output, latent times, and feeding rates. Similar arguments likely explain increased virulence in mite-vectored viruses, and of course have to be studied in the context of evolved defenses, or the lack thereof, in their bee hosts.

(https://www.ncbi.nlm.nih.gov/assembly/GCA_002443255.1). These expanding resources allow the identification of putative *Varroa* orthologs for key arthropod proteins [8••], leveraging years of insights from well-studied insects and mites. Recently, extensive proteomic resources for *V. destructor* were published and used to categorize age and sex-specific gene expression ([9•]; http://foster.nce.ubc.ca/*Varroa*/index.html). Genomic resources will also speed efforts to identify the mechanisms behind key *Varroa* traits, from virulence (Box 1) to acaricide resistance.

Mite–Honey bee interactions

All mite life stages require close interactions with their honey bee hosts ([10] and Figure 1). In the dark hive environment, pheromones and kairomones [11,12•], respectively, and perhaps thermal signals [13], direct and modulate mite behaviors toward each other and their hosts. *Varroa* mites also mimic the chemical profile of their hosts via passive contact, thereby hijacking host communication signals to evade detection [14,15••]. The ability of *Varroa* mites to adopt host chemical signatures and thereby evade detection, even across host species [4•], gives these mites an advantage in the chemical arms race against their hosts.

Phoretic female mites likely take advantage of temporal shifts in honey bee cuticular hydrocarbon profiles to target younger nurse honey bees as preferred hosts over foragers [16]. Nurse bees remain in close proximity to new larval hosts and do not engage in risky flight behavior, and an ability by phoretic mites to target nurses likely increases mite fitness significantly. Disrupting important aspects of the *Varroa* chemical detection system is a frequent theme of mite control [12•,17•]. Once identified, such disrupters will face an evolving counter-attack from the mites themselves. On the host side, there is strong selective pressure to detect *Varroa* directly [18] or via pheromones released by parasitized bees [19•]. For the latter, recent work suggests the physiological and immunological responses of hosts to *Varroa* [20], are similar to responses to viral and *Nosema* infections [21].

Varroa developmental and reproductive physiology

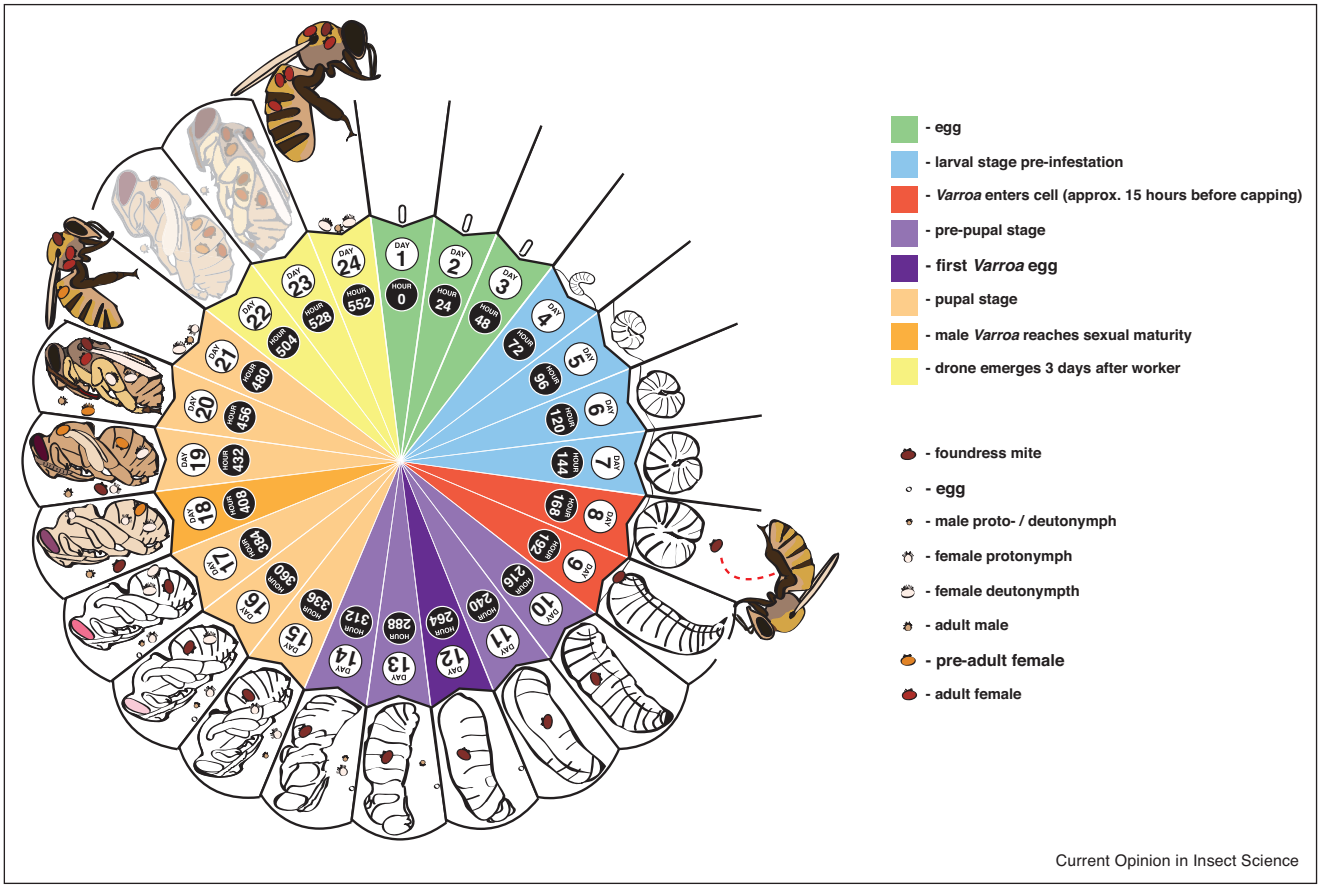
Adult female *Varroa* mites have two distinct life stages, phoretic and foundress. Females in both stages are mated, but reproduction occurs only in the foundress stage, which begins when a female mite finds a suitable host cell and initiates the production of vitellogenin in terminal oocytes. Upon entering a host cell, oviposition occurs sequentially, resulting in a single son and up to 7–9 daughters per foundress, depending on host caste (i.e. worker versus drone; Figure 1). A recent proteomic analysis by Alison McAfee and colleagues [9•] showed profound differences between male and female developmental stages in expression of genes related to cuticle formation, carbohydrate and amino acid metabolism, and vitellogenin protein synthesis, beginning during the late deuteronymph stage. To locate mates, male mites perceive female sex pheromones with tarsal sensory pits; males prefer freshly molted females, which emit a more potent sex pheromone [22]. After copulation, a five-day capitation period is needed prior to egg laying, necessitating a move to a new bee host [23••]. Daughters that mate toward the end of their host's development time therefore require a longer phoretic stage prior to their first round of reproduction. The average time that females remain phoretic is unknown, nor is it known whether a phoretic stage, per se, is necessary as opposed to simply the capitation time period. Phoretic stage mites do show distinct behavioral traits when compared to emerging or reproductive mites [17•].

Recent work explores the chemical cues female *Varroa* mites use to find suitable developing honey bees (brood; [11,12•]. A possible cue may relate to physiological differences between adult workers and brood in their response to parasitism [20]. This cue may also alert phoretic females of prior occupancy of host cells. *Varroa* reproductive success relies on effective coordination between physiological readiness and responsiveness to host cues, likely explaining highly variable levels of reproduction observed for female mites housed *in vitro* [24,25]. When phoretic mites identify and enter host brood cells, a suite of gene-regulation changes occurs. Transcriptomic analyses suggest diverse mechanisms to detect pheromones and other odorants, but a curious lack of expression changes in gene products for odorant binding receptors and co-receptors [26•].

Varroa nutritional physiology

When feeding on bees, mites appear to use a sucking mechanism to extract food from relatively large feeding holes they scrape into the host integument. Feeding behavior can suggest physiology via the number of

Figure 1



Reproductive life cycle of *Varroa* mites, beginning when mated female mites enter the reproductive cell prior to capping. After several days, females lay sequential eggs. Offspring from one or more females mate within the cell and then emerge with worker bees on Day 21 and male (drone) bees on Day 24 of development.

feeding sessions and duration of each [27]. Current observations reveal female mites prefer to feed from the abdomen of host pupae [24]. Additional details of *Varroa* nutritional physiology have come from recent studies of mite-host, worker-host, and drone-host lipid metabolism [28•,29]. Comparing results across these studies indicates the composition of fatty acid profiles of female mites differs depending on whether these mites fed on worker or drone pre-pupal hosts; mites from worker hosts contain greater quantities of short-chain saturated fatty acids and a higher percentage of poly-unsaturated fatty acids [28•]. Trophic changes in fatty acid composition suggest mite physiology includes mechanisms for uptake of specific fatty acids; stearic acid content is amplified in mites from drone hosts, and unique fatty acids were identified from mites [29]. Supporting the strong role of lipids in mite physiology, the most abundant member of the mite proteome is a family of large glycolipoproteins (vitellogenin) involved in lipid transport [9•].

Acaricides and *Varroa* resistance

Thanks to *Varroa*'s grave impact on honey bee health, numerous synthetic acaricides, including organophosphates, pyrethroids and formamidine pesticides have been developed and deployed against mites. Mites have evolved resistance to all of these at some level, although the formamidine Amitraz remains largely effective. Since there is great economic interest in reviving ineffective acaricides and slowing resistance toward others, much effort has been spent identifying mite changes that confer resistance. For example, mutations in *Varroa* sodium channel proteins not surprisingly are linked to resistance to the pyrethroid tau-fluvalinate [30], and metabolic adaptations of *Varroa* esterases may facilitate decomposition of coumaphos, thus accelerating resistance to this compound [31]. Controlled tests indicate resistance to Amitraz and two pyrethroids is widespread in a European population of mites [32] although a mechanism for Amitraz resistance has not yet been described. Price and colleagues used a heterologous functional assay to show

that specific differences in the *Varroa* GABA-activated RDL receptor can explain heightened sensitivity to the commonly used acaricide thymol, and might also be useful in designing novel acaricides that do not impact honey bee hosts [33•]. Optimistically, the >400 million-year divergence time between honey bees and *Varroa* suggests that fundamental differences in their biologies exist, from specific receptors to cell membranes, development and behavior. These differences favor the search for acaricides that have minimal impact on host bees. Organic acids (e.g. oxalic acid) targeting phoretic mites are in wide use but show limited effectiveness during the reproductive months, when most mites are protected in sealed honey bee cells [34]. The mode of action of organic acids against *Varroa* may be mechanical in nature, and most are simple molecules, thus the chances are low for *Varroa* gaining physiological resistance to their effects [35]. *Varroa* may, however, exhibit behavioral resistance to organic-acid varroacides such as oxalic acid. In this case, resistance might take the form of female mites reducing the time spent in the phoretic stage, thus minimizing chance of exposure. Future *Varroa* controls should be vetted not only for their effects on honey bees, but also for whether and how mites will evolve resistance against these controls. An integrated approach of alternating use of different varroacides should also help reduce chances of *Varroa* gaining resistance to any single compound.

Insights into the *Varroa* microbiome

Varroa mites share microbes with their bee hosts and also appear to harbor a set of unique microbes. Of the shared microbes, the most impactful are RNA viruses that infect honey bee hosts. Transmitted to bees while feeding, and picked up by naïve mites the same way, viruses in the Deformed wing virus group are especially damaging to bees and are the subject of urgent research. These viruses continue to expand in range and in their impacts on honey bees and other insects [36••,37•], largely because of their connection to *Varroa* mites. Indeed, restriction of these viruses would likely make *V. destructor* a more benign honey bee pest overnight. It is also conceivable that *Varroa* mites carry actively replicating viruses that are harmful to mite health, and that could thereby be exploited for mite control. In this scenario, honey bees could serve as an unaffected reservoir to deliver viruses into *Varroa mites*, in many ways a reversal of the current system. *Varroa* also harbor bacteria and fungi and the former have been studied serendipitously during genomic sequencing efforts [7] along with targeted searches using amplicon-based sequencing [38•,39]. Several strains of the bacterium *Bacillus thuringiensis* isolated from *Varroa* show promise as biocontrol agents [40]. Fungal associates of *Varroa* have not yet received the same genetic scrutiny and there is a great need to do so, since there have been successes using known entomopathogenic fungi to infect *Varroa* populations [41]. One extension of this work has been to isolate fungal virulence factors that show promise as stand-alone acaricides [42•]. Protists associated with *V. destructor* are poorly described, although extensive transcriptomic and genomic analyses of these mites should identify mite-specific protists. The Holobee Database [43] was developed to compile known and sequence-inferred microbes in both honey bees and associated organisms, including *Varroa*. The hope in this emerging field will be to find critical microbes confined to *Varroa* that might be used to either control mite populations or reduce the capacities to transmit RNA viruses.

Prospective gene-based controls

While there have been significant genetic advances for *Varroa*, along with insights into mite development and reproduction, few studies have married these concepts to propose novel gene-inspired controls. One exception comes from the field of RNA interference (RNAi), where several studies have shown that RNAi functions in *Varroa* and transcript knockdowns can be used to infer protein function. First, Campbell and colleagues showed that the levels of the important enzyme glutathionine S-transferase could be reduced by RNAi, albeit only by injecting mites or submerging them in a concentrated solution of triggering double-stranded RNA [44•]. Further, Singh and colleagues [17•] showed that knockdowns of a transcription factor led to a predicted increase in the key reproductive protein vitellogenin, mimicking the physiological change tied with shifting to a reproductive mode. Delivery challenges were given a boost by research showing dsRNA could, in principle, be delivered to mites by simply feeding their bee hosts [45•].

RNAi is not the sole genome-enabled strategy aimed at *Varroa* mites. Peptidomimetic regulators of *Varroa* signaling are promising and candidate targets for this strategy are suddenly plentiful [46]. In addition, simply using genetic insights to decrease the search time for discovering exploitable weaknesses in *Varroa* biology will help identify control strategies unlikely to impact bee hosts [47•]. These searches can leverage the dozens of other genomes available for better-studied organisms, now that mites have a well-described genome and predicted gene set.

Conclusions

Genomic resources for *Varroa* paired with experimentation and tools, such as proteomics, are illuminating *Varroa* biology and the key traits used by these mites to identify and exploit their bee hosts. Gene-based insights should provide specific means for decreasing mite numbers and their impacts. *Varroa* mites are by no means the only threats to honey bees, whose colonies are at risk from other parasites, pesticides, and poor resources in their environment. Nevertheless, any steps than can be taken to reduce mite impacts will surely improve the fate of the world's most important agricultural pollinator.

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