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Forum

Are Dispersal Mechanisms Changing the Host–Parasite Relationship and Increasing the Virulence of *Varroa destructor* (Mesostigmata: Varroidae) in Managed Honey Bee (Hymenoptera: Apidae) Colonies?

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Abstract

Varroa (*Varroa destructor* Anderson and Trueman) are a serious pest of European honey bees (*Apis mellifera* L.), and difficult to control in managed colonies. In our 11-mo longitudinal study, we applied multiple miticide treatments, yet mite numbers remained high and colony losses exceeded 55%. High mortality from varroa in managed apiaries is a departure from the effects of the mite in feral colonies where bees and varroa can coexist. Differences in mite survival strategies and dispersal mechanisms may be contributing factors. In feral colonies, mites can disperse through swarming. In managed apiaries, where swarming is reduced, mites disperse on foragers robbing or drifting from infested hives. Using a honey bee–varroa population model, we show that yearly swarming curtails varroa population growth, enabling colony survival for >5 yr. Without swarming, colonies collapsed by the third year. To disperse, varroa must attach to foragers that then enter other hives. We hypothesize that stress from parasitism and virus infection combined with effects that viruses have on cognitive function may contribute to forager drift and mite and virus dispersal. We also hypothesize that drifting foragers with mites can measurably increase mite populations. Simulations initialized with field data indicate that low levels of drifting foragers with mites can create sharp increases in mite populations in the fall and heavily infested colonies in the spring. We suggest new research directions to investigate factors leading to mite dispersal on foragers, and mite management strategies with consideration of varroa as a migratory pest.

Key words: honey bee virus, feral colony, swarming, migration, varroa–virus complex

Varroa, *Varroa destructor* Anderson and Trueman (Mesostigmata: Varroidae), is an external parasite of both larval and adult honey bees *Apis mellifera* L. (Hymenoptera: Apidae). The mite is a serious pest of European honey bees inflicting more damage and economic costs than all other apicultural diseases (Genersch 2010, Rosenkranz et al 2010). Varroa is an invasive species that originated in Asia where *Apis cerana* F. was the host. The mite does little harm to *A. cerana* and maintains a stable host–parasite relationship largely because the mite reproduces only in drone brood that comprises <5% of the colony's brood population (Fuchs 1990). When mites attempt to infest worker brood cells, the parasitized pupa and mites are removed by adult bees exhibiting "hygienic behavior" (Peng et al. 1987). Adult bees also remove and kill mites on nestmates ("grooming behavior"). In the 1950s, varroa shifted hosts from A. *cerana* to the European honey bee (*A. mellifera*). In *A. mellifera*, varroa parasitizes and reproduces in both worker and drone brood. Adult bees also can be parasitized, but varroa are seldom found on queens.

A comprehensive description of the varroa lifecycle is provided in Rosenkranz et al. (2010), so only a brief overview is provided here. Varroa reproduce in capped worker and drone brood cells. Mature female mites (called mother mites or foundresses) enter cells just prior to capping. The foundress starts feeding on the brood within 6 h of the cell being sealed, and feeding occurs regularly thereafter (Donzé et al. 1996). The site on the larva where the foundress pierces the cuticle to feed becomes the feeding area for her offspring. The first egg laid by the foundress develops into a male. The second egg develops into a female mite that mates with the male. The foundress mite feeds on the developing larva, and can transmit several different viruses in the process. In worker brood, foundress mites produce one to two mated daughter mites. In drone brood, which takes longer to mature, two to three mated daughters can be

produced. When the bee emerges from the capped cell, the foundress mite and her daughters emerge with it and attach to adult bees as "phoretic mites." Most commonly, phoretic mites attach to young worker bees tending developing brood (i.e., nurse bees). Mites discriminate between nurse bees and older bees by the differences in their cuticular hydrocarbon profiles (Del Piccolo et al. 2010, Cervo et al. 2014). Nurse bees are the target of phoretic mites because the bees remain in the brood area and can serve as a vehicle to transport mites to brood cells. Phoretic mites can feed on adult bees, but when a brood cell of suitable age is found, the mite will detach and enter the cell to reproduce.

The reproductive rates of varroa are relatively low, with each foundress mite producing one to three offspring. Colonies that have low mite infestations should take several years before populations reach a point where they threaten colony survival (Genersch 2010, Rosenkranz et al. 2010). In recent years, however, mite populations have grown at rates that far exceed those expected from reproduction alone (DeGrandi-Hoffman et al. 2014, 2016). The rapid growth of varroa populations particularly in commercial colonies has caused beekeepers to apply up to seven miticide treatments each year. In spite of this, in 2014–2015, colony losses in the United States were 40% (Seitz et al. 2015), and preliminary results indicate that they may have reached 44% in 2015–2016 (https://beeinformed.org/results/colony-loss-2015-2016-preliminary-results/) (accessed 8 April 2017). Varroa was a major factor in the colony losses.

Here, we show the challenges of controlling varroa in commercial apiaries by reporting data collected from a longitudinal study that compared colony growth and survival with different numbers of miticide treatments throughout the year. We then describe dispersal mechanisms of varroa within the framework of a parasite that has shifted from survival in widely spaced feral honey bee colonies to managed apiaries with high concentrations of hives. We also discuss the association of the mite with honey bee viruses because they are a compounding factor in the devastation varroa causes in managed colonies. The varroa–virus association also might be a contributing factor in the mite dispersal mechanisms that are unique to managed apiaries. Evidence of possible changes in the mite's dispersal strategies is provided from simulations generated by a honey bee colony–varroa population dynamics model (VARROAPOP; DeGrandi-Hoffman and Curry 2004). Based on the results from field studies and model simulations, we suggest future directions for research and development of varroa management strategies to reduce colony losses through a more in-depth understanding of varroa dispersal mechanisms in managed hives.

Varroa Populations in Migratory Commercial Colonies

We measured the growth of varroa populations in an 11-mo longitudinal study in commercial apiaries. The study began in 2015 when hives that were in California for almond pollination were moved in late March to Texas. Sixty colonies were split in April to create two hives with 8–10 frames of bees and brood (120 colonies total). A laying European queen purchased from Olivarez Honey Bees Inc. (Orland, CA) was added to the queenless colony in a self-releasing cage ~48 h after making the split. The hives remained in Texas apiaries until June, and then were moved to North Dakota until November. The colonies were moved back to Texas in November, and overwintered there. In January, hives were moved to California for almond pollination.

The 120 hives were divided into two treatment groups. A group of 60 colonies received miticide treatments only in September and again in November (fall only treatment). A second group of 60 colonies was treated in May and June to maintain low mite numbers throughout the summer, and then again in September and November (spring and fall treatment). Colonies were treated with HopGuard II (BetaTec Hop Products, Washington, DC) for all but the November treatment when Apivar (Arista LifeScience America, New York) was applied. The active ingredient in HopGuard II is a potassium salt of beta acids from hop (*Humulus lupulus* L.) plants and is considered a "soft" mite treatment (DeGrandi-Hoffman et al. 2012). Apivar has amitraz as the active ingredient and is a "hard" mite treatment. All miticide applications were made in accordance with the manufacture's instruction on the package label. The effects of the miticide on the varroa population were measured by counting the number of mites that dropped on to a sticky board placed on the bottom board of the colony in the 48 h before (pretreatment) and after the application (posttreatment). Mites were counted using the stratified sampling technique developed by Ostiguy and Sammataro (2000).

Based on the methods for colony measurement described in Delaplane et al. (2013) and DeGrandi-Hoffman et al. (2014), colonies in the "fall only treatment" group grew to an average (± SE) of 16 ± 0.5 frames of bees and brood (40,470 ± 1,140 adult bees) by May. Colonies in the spring and fall treatment group grew to 17 ± 0.2 frames of bees (42,300 ± 589 adults). Prior to the first miticide application in May, the number of mites on sticky boards (mite drop) after 48 h in both treatment groups was <2 mites per hive (Fig. 1). After the miticide application, treated hives had an average mite drop of 13.5 ± 1.3 mites over the 48-h posttreatment interval. A second HopGuard II treatment was applied in late May, and 6.3 ± 0.8 mites dropped per colony during the 48-h posttreatment interval. During the same period, fall only treatment colonies dropped 2.3 ± 0.3 mites in 48 h.

In September, when hives were in North Dakota, colonies in both groups were similar in size and averaged 15 frames of bees (fall only treatment: 37,089 ± 664, spring and fall treatment: 38,298 ± 607). Pretreatment mite drop averaged 267 ± 30 mites per hive in the fall only treatment and 72 ± 8 in colonies previously treated in the spring. All colonies received a HopGuard II treatment. Forty-eight hours after treatment, mite drop in the fall only treatment averaged 2,774 ± 213 mites per hive. Colonies previously treated in the spring had an average mite drop of 1,300 ± 100 mites per hive. Although miticide applications were applied in September, and from the mite drops, appeared to be effective, colony survival declined from 75% (May–September) to 37% between September and November in fall only treatment hives (Fig. 2). Survival of colonies that were treated previously in the spring went from 76% in September to 60% in November. Based on a Fisher's exact test, the survival rates between the treatment groups were not significantly different ($P = 0.15$).

All colonies were treated with Apivar in November, and dropped an average of about 100 mites in the 48-h posttreatment period. Although two fall treatments were applied to all colonies, only 33% of those in the fall only treatment and 45% in the spring and fall treatment were still alive in January. Again, there was no significant difference in survival between treatments (Fisher's exact test, $P = 0.40$).

Varroa Survival Strategies

The success of a parasite depends on both its efficiency in exploiting its hosts, and the ability to disperse when host conditions become

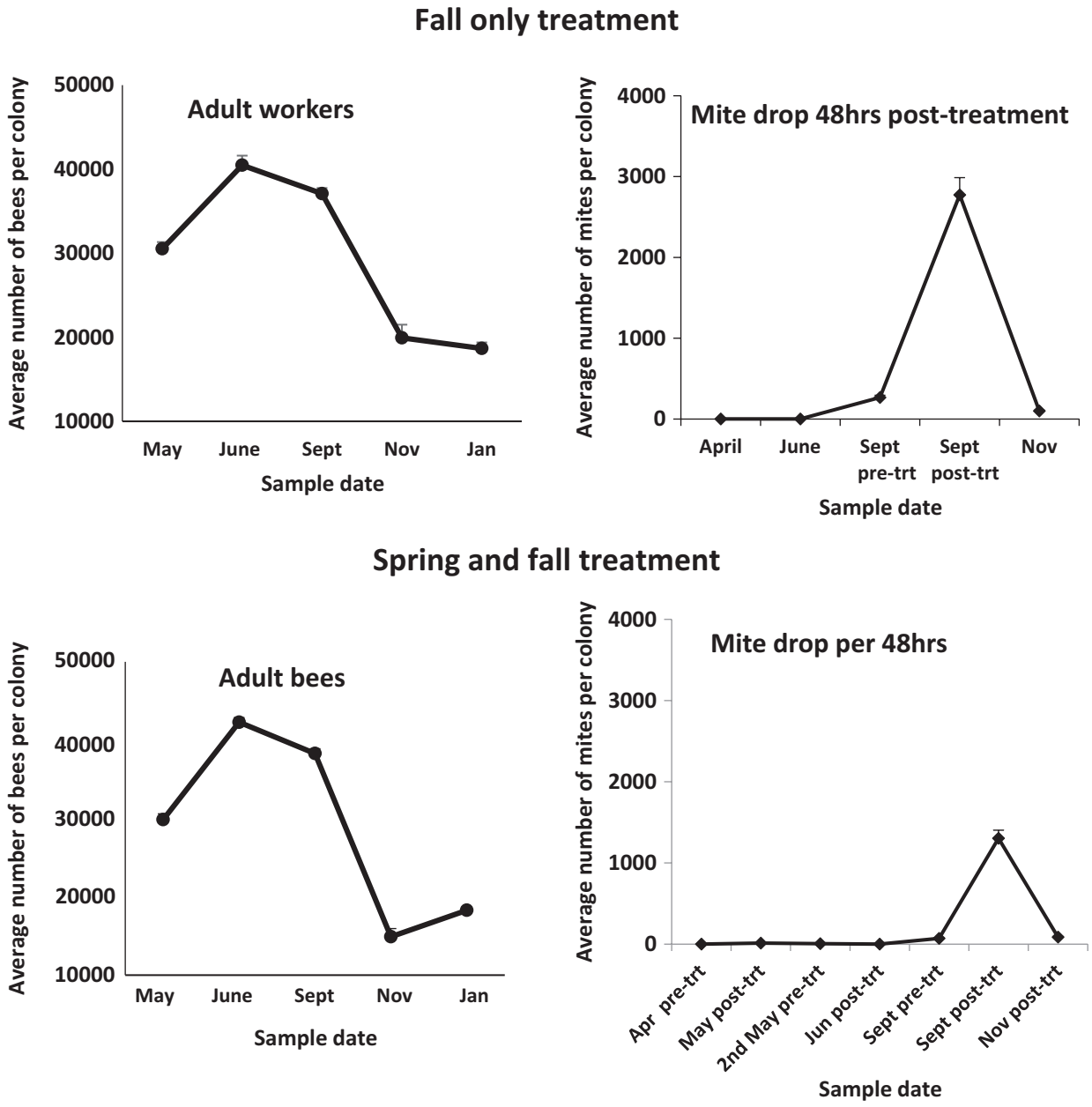

Fig. 1. Average colony sizes and 48-h mite drop on sticky boards of colonies in a longitudinal study to measure colony growth and mite populations in commercial honey bee colonies. Colonies were treated with HopGuard II in the May–June and the September treatments. In November, colonies were treated with Apivar. Mite drop was counted 48 h before and after mite treatments. In the "Fall only treatment," mite drop was counted during the pretreatment interval for the Spring and fall treatment.

unfavorable. Dispersal to a more suitable host is a crucial step in the life cycle of parasites (Combes 2005). Varroa initially evolved its relationship with feral honey bees living in natural settings such as tree cavities. Feral colonies often exist at low densities of 1 colony per km^2, with an average nearest-neighbor distance of 850 m (Seeley 2007, Seeley and Smith 2015). In these settings, varroa populations that cause colonies to collapse would perish with the bees if the colony was not discovered and robbed prior to its death.

There are reports of feral colonies persisting for years despite being infested with varroa (Le Conte et al. 2007, Seeley 2007). These colonies represent survivor populations that, through selective pressures by the mites and viruses, may have developed intrinsic resistance mechanisms (Loftus et al. 2016). Another way that varroa-infested feral colonies might survive, however, is through swarming (Loftus et al. 2016). At low varroa populations, the mite could coexist with the bees and inflict minimal damage, so that colonies would expand in the late spring and early summer. The growth of feral colonies is limited by the size of the nest cavity. When there is no longer space for expansion, colonies will swarm (Simpson and Ridel 1963). Colonies that swarm can lose an estimated 35% of the phoretic mite population (Loftus et al. 2016). There also is a break in the brood cycle when colonies swarm. Workers stop feeding the queen and she does not lay eggs, while colonies prepare to swarm (Michener 1974; see Grozinger et al. 2014). When the colony's

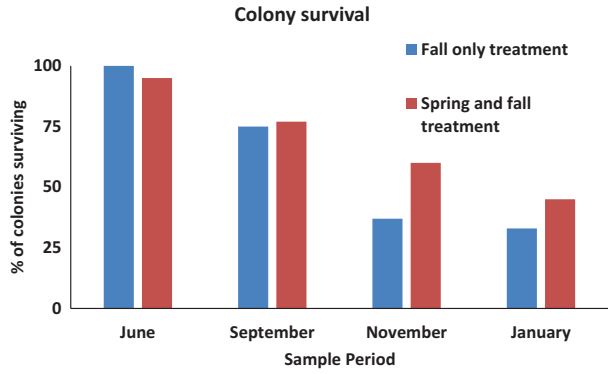

Fig. 2. Percentage of colonies surviving from June 2015 to January 2016 under two different varroa treatment schedules. Miticides were applied to "fall treatment only" hives in September and November, and in "spring and fall treatment" hives in May, June, September, and November. The survival rates of colonies in November did not differ between those treated in the spring and fall, and those treated in the fall only (Fisher's exact test, $P = 0.15$). The percentage of colonies that survived through January also did not differ between treatments (Fisher's exact test, $P = 0.40$).

Table 1. Initial conditions common to all VARROAPOP simulations

Initial condition parameter	Initial condition value	Reference
Queen egg-laying potential	2,500 eggs per day[a]	Defined initial condition
Forager lifespan	14 d	Defined initial condition
Mated daughter mites per foundress (single infestations)[b]	Worker cells—1.5 Drone cells—2.7	Martin 1994, 1995
% of successful reproduction events per brood cell invasion (single infestations)	Worker cells—80% Drone cells—90%	Defined initial condition

A complete description of the VARROAPOP model is available in DeGrandi-Hoffman and Curry (2004).

[a] Queens can lay 2,500 eggs under optimum conditions for weather, daylength, and colony size. On each day of the simulation, queens lay fewer than 2,500 eggs per day if conditions deviate from optimal. For a complete description of factors affecting eggs laid per day, see DeGrandi-Hoffman et al. (1989). This egg-laying potential generated an average of $1{,}259 \pm 37$ new eggs per day during the height of brood rearing season (1 May–1 September).

[b] Reproductive success and the number of mated daughter mites decreases in worker and drone cells when cells are multiply infested with foundress mites. Multiple infestations occur in nature and in simulations when the number of phoretic mites exceed that of brood cells of suitable age to infest. Reductions in mite reproduction with increasing numbers of foundress mites in worker and drone cells are reported in DeGrandi-Hoffman and Curry (2004).

Table 2. Initial conditions for VARROAPOP simulations

Simulation type	Parameter	Value for initial condition
Swarming and colony survival	Start date	April 1
	Weather conditions	Aurora, Il—2000–2005
	Eggs	4,000
	Larvae	3,000
	Capped brood	2,000
	Adult workers	12,500 (5 frames of bees)
	% Adults with phoretic mites	3%
	% Capped brood with mites	5%
Mite migration	Start date	May 1
	Weather conditions	Overwinter confinement Aurora Il 2001–2002 Active overwinter Tucson, AZ 2008–2009
	Eggs	0
	Larvae	0
	Capped brood	0
	Adult workers	9,000
	% Adults with phoretic mites	0.3
	% Capped brood with mites	0

queen leaves with the swarm, the nest is left with a virgin queen, so there is no new brood until the virgin queen mates and starts laying eggs (about 10 d after emergence; Winston 1987). The combination of the break in brood cycle that creates a break in mite reproduction, and loss of mites with the swarm might keep mite levels low and enable colonies to coexist with varroa indefinitely.

To test if colonies could theoretically coexist with varroa if there was yearly swarming, we conducted VARROAPOP simulations of infested colonies with and without swarming. All simulations had the same initial colony conditions and were run for a 5-yr period (Table 1). In these simulations, 3% of adult bees had phoretic mites and 5% of sealed brood were infested with mites at the start of the simulation (Table 2). Swarming always occurred on 15 June as did queen replacement in simulations without swarming. In simulations with swarming, egg laying stopped for 2 d before the swarm departed (Michener 1974) and did not resume until 10 d after the swarm left. These conditions simulate the approximate period needed for the new queen to mate and begin egg laying. Simulations without swarming included a 10-d period without egg laying beginning on 15 June to simulate loss of the laying queen and the maturation and mating period of a new queen. In the simulations with swarming, colonies lost an average of 33.4% of the adult bees and 35% of the phoretic mite population each year when the colony swarmed. The swarms issued by the colonies ranged from 8,000–11,000 bees depending upon the year and colony size at the time of swarming.

In simulations where colonies did not swarm, the mite population increased exponentially by late summer of year 2, and the colony died the following spring (Fig. 3). In contrast, colonies survived and mite populations remained at low levels for the 5-yr period when there was swarming. The ability of colonies to coexist with mites for long periods is supported by field observations of feral bees that nest in small cavities and swarm regularly. The colonies have fewer varroa, less disease, and higher survival rates than colonies that did not swarm (Seeley and Smith 2015, Loftus et al. 2016). Apparently, smaller nest cavities and more frequent swarming of feral colonies contribute to their persistence without miticide treatments. This persistence might be interpreted as mite resistance, but in fact may be owing to cavity size and resource availability that stimulates nest expansion and culminates in swarming.

In contrast to conditions in feral colonies, managed apiaries often have high numbers of closely spaced colonies. Beekeepers reduce swarming by adding more wax comb to hives with expanding populations or by splitting populous colonies. The conditions in managed apiaries that are in direct contrast to those experienced by feral colonies perhaps have led to the selection of other dispersal

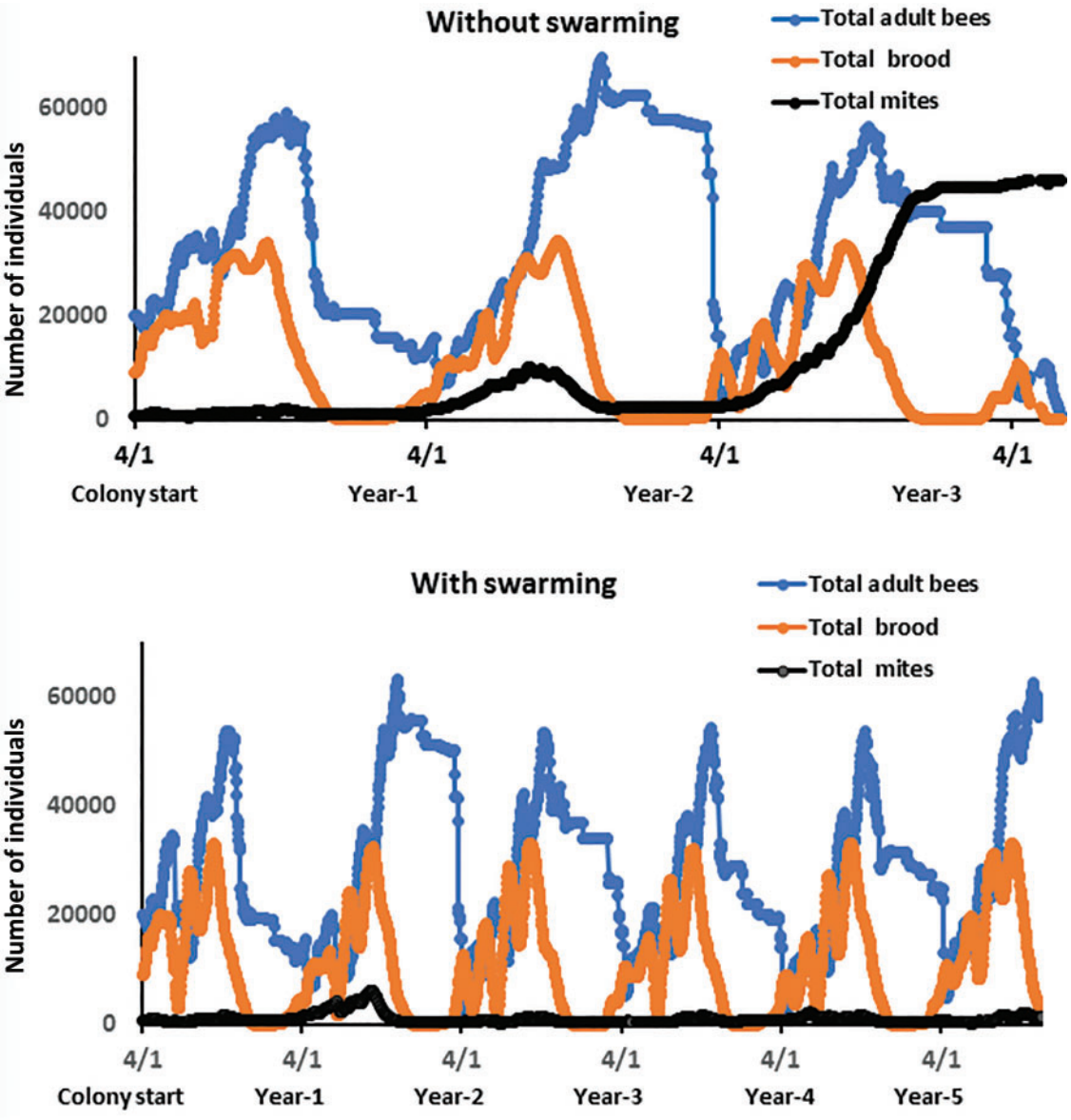

Fig. 3. Results from VARROAPOP simulations of colony and varroa population growth with and without yearly swarming. "Total adult bees" includes workers and drones. "Total brood" is the sum of eggs, larvae, and pupae, and "Total mites" is phoretic mites and mites infesting brood cells. Both groups of simulations had identical initial conditions. In simulations with swarming, the event occurred on June 15 each year. In simulations without swarming, the queen was replaced on 15 June, but there was no decrease in the adult bee population or reduction in mite numbers as occurs during swarming.

mechanisms for varroa and the viruses they transmit. In the wild, the death of a varroa-infested colony probably means the demise of both the bees and the mites. In managed apiaries, though, the mites could survive because foragers from stronger colonies rob those weakened from varroa. Mites can attach to the robbers and be transferred to other colonies. Additionally, low level drifting of foragers with mites occurs particularly in the fall (Sakofski et al. 1990, Frey and Rosenkrantz 2014, DeGrandi-Hoffman et al. 2016). Both the transfer of mites on to robbing bees and the drifting of foragers with mites may be dispersal mechanisms adapted for apiary settings that have caused serious challenges for mite control.

The Varroa–Virus Complex

Varroa are efficient vectors of several honey bee viruses. The most common viruses transmitted by varroa have single-stranded RNA genomes and belong to species included in the families Dicistroviridae (Acute bee paralysis virus, ABPV; Black queen cell virus, BQCV; Israeli acute paralysis virus, IAPV; Kashmir bee virus, KBV) and Iflaviridae (Deformed wing virus, DWV; Sacbrood bee virus, SBV) in the order Picornavirales (Chen and Siede 2007, Runckel et al. 2011). Honey bee colonies typically are infected with multiple viruses (DeMiranda et al. 2010, Carrillo-Tripp et al. 2015). Prior to the introduction of varroa, virus infections did not pose serious health problems in honey bees (Genersch and Aubert 2010). However, as varroa populations increase in colonies, so do virus titers (Francis et al. 2013). Therefore, it is not surprising that previously asymptomatic virus infections become acute, overt infections in colonies with high varroa infestations (McMenamin and Genersch 2015).

Of the viruses transmitted by varroa, perhaps the most serious is DWV. The virus is vertically transmitted via eggs and semen and is

present in most members of a colony (Chen et al. 2006, Yue et al. 2007, DeMiranda and Fries 2008). Before varroa was found in colonies, wing deformities were rarely seen. When deformed wings were seen in bees emerging from cells infested with varroa, the damage was attributed to parasitism and hemolymph deprivation (De Jong et al. 1982, Koch and Ritter 1991, Marcangeli et al. 1992). However, studies found a direct correlation between DWV titers and the symptoms associated with the virus that include deformed wings, paralysis, learning deficits, and a drastically shortened life span (Yang and Cox-Foster 2007, Bailey and Ball 1991).

The learning deficits associated with DWV infections might be related to the presence of the virus in regions of the honey bee brain, including optic and antennal neuropils and mushroom bodies (Shah et al. 2009). The virus actively replicates in these brain regions and may cause impaired vision and compromised olfactory processing that may ultimately affect flight behavior, homing performance, and perception of odorants. Laboratory studies using controlled infections with DWV support some of these possibilities in that injecting DWV into foragers causes impaired sensory responsiveness, memory formation, and associative learning (Iqbal and Mueller 2007).

The increase in symptomatic DWV infections in varroa-infested colonies might be related to a mutualistic relationship between DWV and varroa that improves parasite feeding and virus transmission and replication (DiPrisco et al. 2016). The relationship begins when DWV is vectored by varroa feeding. The virus adversely affects humoral and cellular immune responses by interfering with antiviral barriers under the Toll pathway. Specifically, NF-κB signaling (a component of the innate immune system) is affected, and this can have a multifaceted influence on the immune response (Nazzi et al. 2012). For example, when the foundress mite pierces the cuticle and begins feeding on the developing bee, the wound that is created activates NF-κB for clotting and melanization (Lemaitre and Hoffmann 2007). Suppression of NF-κB signaling by DWV facilitates mite feeding and possibly reproduction by preventing hemolymph clotting at the feeding site (DiPrisco et al. 2016). Without interference from clotting, mites perhaps transmit virus more successfully. Increased success in mite reproduction and virus transmission ultimately generate a loop of escalating stress on honey bee immunity and impaired behavioral function that over time negatively affects colony health and survival (DiPrisco et al. 2016). This could explain why varroa and DWV are a leading cause of colony loss over the winter (Genersch et al. 2010).

Workers emerging with deformed wings are common in colonies heavily infested with varroa, particularly in the fall, and are a harbinger of colony loss over the winter. However, colony death over the winter is ultimately a losing strategy for the mite and the virus, as both would perish with the bees. Deformed wing virus and Varroa are ubiquitous in managed colonies, so other mechanisms must be in place to disperse mites and virus before colonies collapse in the winter.

Mite Dispersal

Though mites can disperse on foragers that rob collapsing colonies, mites also can spread if they attach to foragers that drift into hives other than their own (Sakofski et al. 1990, Greatti et al. 1992). Most drifting occurs among colonies in the same apiaries, but foragers with mites also can drift into colonies from hives outside of those apiaries (Frey et al. 2011). Drifting occurs throughout the spring and summer, but the frequency of drifting foragers with mites increases in late summer and fall when varroa and virus titers are at the highest levels (Frey and Rosenkrantz 2014, DeGrandi-Hoffman et al. 2016).

During our sampling of colonies from July to November in Tucson, AZ, we found an increase in foragers with mites after August (DeGrandi-Hoffman et al. 2016). However, we did not detect high percentages of foragers with mites during any sampling interval, as would be expected if a portion of the foraging population were robbing a heavily infested colony. Instead, we detected a constant low-level influx of mites that over time perhaps, could increase the varroa population to levels significantly greater than those generated from reproduction alone. To test this, we ran VARROAPOP simulations using field data from a study reported in DeGrandi-Hoffman et al. (2016). In this study, colonies were established in May from 3-lb packages of bees. The proportion of foragers with mites was estimated from weekly field samples at the entrances of 20 hives beginning on 11 August and continuing until 24 November. During this time, the percentage of foragers with mites captured during a 3-min sample ranged from 0.2% in August to 3% in November. A range of 85–150 foragers was captured during each sample period. To estimate the total number of mites entering colonies each day, we used data on the number of foragers captured during each sampling interval, the proportion of foragers with mites, and the number of hours of foraging per day based on weather conditions. We approximated the total number of mites entering colonies on foragers from August to November by summing the daily estimates ($\sum$ [(foragers/minute) * (total minutes in the foraging day) * (mites/forager)]). We estimated 1,800 mites per hive entering on foragers from August through November.

Colony sizes and varroa levels measured at the start of the field study were entered into simulations as initial conditions (Table 2). Simulations were conducted under two types of weather conditions. The first set of conditions simulated "winter confinement," where bees did not rear brood or forage from 22 November to 24 January owing to low temperatures and short daylength (DeGrandi-Hoffman et al. 1989). In "active over winter" conditions, bees reared brood and foraged each day owing to higher temperatures and longer daylengths. The 1,800 mites were added to the phoretic population from August through October in simulations with winter confinement, and August through November in "active over winter" simulations. The rate that mites were added during the 3- or 4-mo period was expressed using an exponential curve, with the greatest number of mites entering colonies in September and October in simulations with winter confinement, and October through November in simulations where colonies were active over winter. Simulations were run with and without the addition of mites (i.e., with and without mite migration).

In simulations without mite migration, varroa numbers remained low throughout the fall and winter, and rose slightly in the spring. However, when there was mite migration, varroa levels rose throughout the fall and increased sharply in the spring. Colonies that were confined over the winter had small heavily infested populations in the spring that would probably not survive (Fig. 4). Spring populations in colonies that were active over the winter were larger than when bees were confined. However, the increase in varroa owing to migration resulted in heavily infested hives in the spring that would require late winter or early spring miticide applications to prevent colony loss.

Conclusions and Future Directions

Selection pressures that shape varroa population dynamics and dispersal strategies differ greatly between infestations in widely

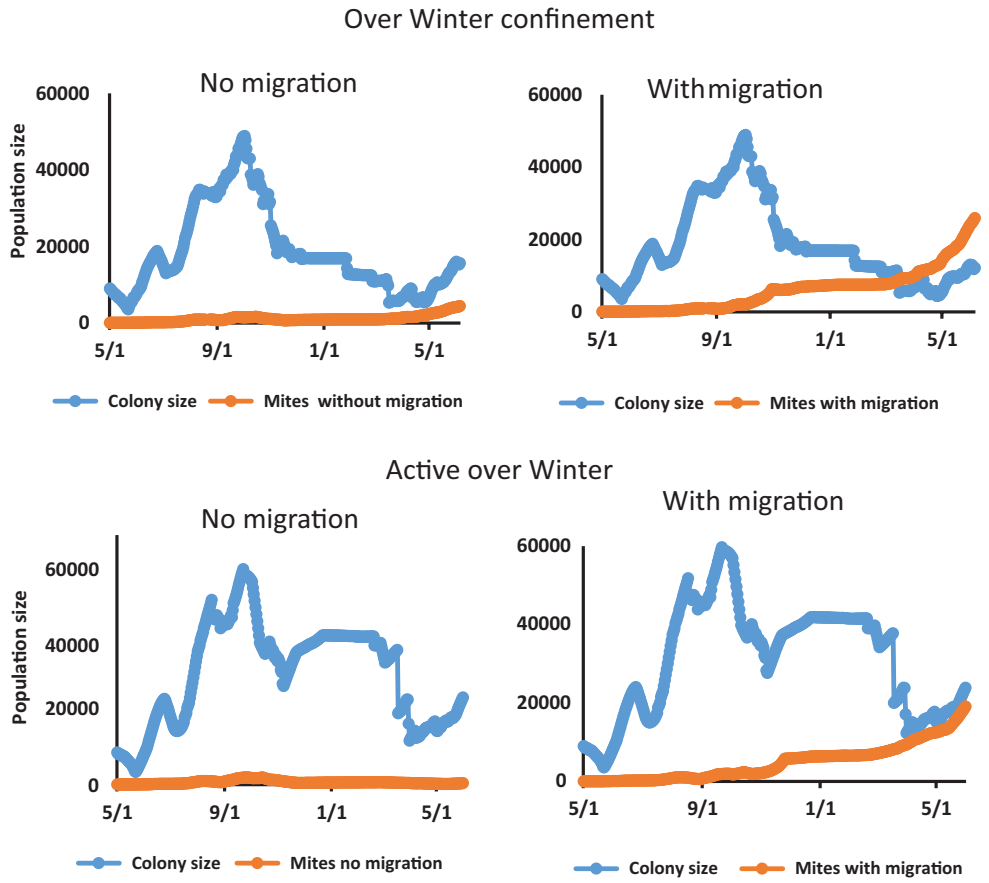

Fig. 4. Predicted honey bee colony and varroa populations with and without mite migration generated from a honey bee colony-varroa population dynamics model (DeGrandi-Hoffman and Curry 2004). Initial colony and mite population sizes and estimates of mites migrating into colonies on foragers are based on field data reported in DeGrandi-Hoffman et al. (2016). Model simulations were run using two types of weather conditions: "winter confinement" where bees did not forage from mid-November through mid-March or rear brood (22 November–24 January), and "active over winter" where bees reared brood and foraged over the winter.

distributed and often well-hidden feral colonies, and those in apiaries with numerous hives. In feral colonies, if mite numbers reach levels that severely weaken the colony, bees will not swarm and the mite dispersal strategy is undermined. If a feral colony dies, the mites risk dying with it. In apiaries, high mite numbers that weaken colonies activate the dispersal mechanisms associated with robbing. Varroa dispersal from colonies that are not collapsing also might be occurring with drifting foragers that are carrying mites. Viruses that varroa transmit may mediate this manner of dispersal by the effects they have on the physiology and behavior of bees.

A flow of events that might lead to mite dispersal on drifting foragers based on findings from studies of the varroa–virus complex and the effects on worker bees is shown in Fig. 5. The dispersal strategy might begin with virus titers that increase with mite numbers in the spring and summer and peak in the fall. Workers parasitized during development and infected with virus will attract foundress mites when they emerge because the workers have a cuticular hydrocarbon profile of a nurse bee (Del Piccolo et al. 2010). However, stress from parasitism and viral infection can cause young workers to forage precociously (Natsopoulou et al. 2016). Precocious foragers are more likely to drift (Ushitani et al. 2016) as are those infected with DWV, as the virus can compromise cognitive function (Iqbal and Mueller 2007). These conditions might increase the chances that the forager will drift into another hive and disperse the mite and virus they are carrying.

The ability of parasites, virus, and other pathogens to enhance their transmission and dispersal by manipulating host behaviors is well documented in plants and animals (Heil 2016). For example, viruses can induce changes in infected plants and affect aphid feeding behavior to enhance virus acquisition and spread to uninfected plants (Carmo-Sousa et al. 2016). The baculovirus (*Lymantria dispar* nucleopolyhedrovirus) enhances its dispersal by manipulating the behavior of infected European gypsy moth (*Lymantria dispar* (L.)) (Lepidoptera, Erebidae) larvae causing them to climb to the top of their host trees. Infected larvae liquefy on the treetops and release millions of infective virus particles into the air spreading the virus throughout the forest canopy (Hoover et al. 2011).

Although there is no evidence that varroa and the virus they transmit manipulate honey bee behavior to enhance dispersal, studies could be conducted to test for this relationship. Experiments using the techniques described in Cervo et al (2014) where mites can choose to attach to nurse bees or foragers could be extended to include comparisons of virus titers and cuticular hydrocarbon profiles in foragers that were chosen by mites versus those that were not. Additionally, the age of first foraging for bees parasitized during development and marked at emergence could be compared with unparasitized workers to determine if there is an increased likelihood of precocious foraging in parasitized bees and if this is affected by virus titers. Comparing numbers of marked bees from the two groups (parasitized and unparasitized) recovered in other colonies in

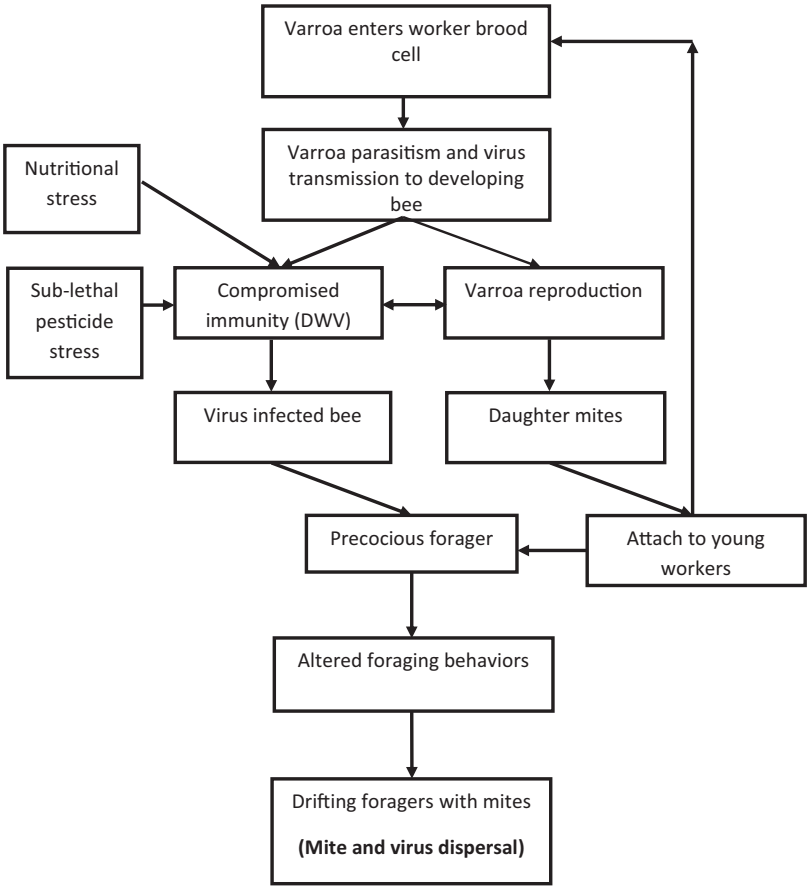

Fig. 5. Conceptual framework for interactions of varroa and deformed wing virus (DWV) that leads to mite and virus dispersal on drifting foragers.

an apiary could determine if there is a greater frequency of drift for parasitized versus unparasitized foragers. Whether the effects of parasitism and virus-induced immunosuppression can be mitigated with improved nutrition also could be investigated, because the nutritional quality of protein sources can affect virus titers and precocious foraging (Toth and Robinson 2005, DeGrandi-Hoffman et al. 2010, DeGrandi-Hoffman and Chen 2015).

Varroa control in managed hives is increasingly difficult, and perhaps not possible with current commercial beekeeping practices. When varroa first arrived in the United States, two well-timed miticide applications (spring and late summer or early fall) could keep mite numbers at levels where they did not threaten colony survival. Now, five to seven miticide applications are needed and colony losses still average >30%. A reason that mites cannot be controlled without repeated miticide treatments is that high densities of colonies in apiaries provide an ideal setting for mite dispersal among hives. The results from the longitudinal study reported here provide a glimpse of what varroa is doing to managed colonies. The apiaries that included our treatment groups had hundreds of hives. If our mortality rates were representative of those in colonies not included in our study, about 25% of the hives died from June to September (Fig. 2). High fall mite numbers in our colonies that were lightly infested in the spring could have been from the dispersal of mites from colonies that had collapsed. September treatments could not save many of our colonies from perishing, indicating that the damage from the mites and virus infections could not be reversed with fall-only miticide treatments. The colonies we lost would themselves become a source of mites dispersing to other hives and adding to their overwinter varroa population.

To improve varroa control and increase colony survival especially over winter, integrated pest management (IPM) strategies need to be developed that treat varroa as a migratory pest. The strategies should incorporate fundamental components of IPM focusing on sustainable control methods that combine resistance management through the rotation of miticides, use of mite-resistant lines, and modifications in beekeeping practices (Delaplane et al. 2005). In addition, the basic principles for managing migratory pests (Pedgley 1993) such as population control on an area-wide basis, anticipation of migratory events, proactive implementation of control strategies, and frequent sampling also should be applied to varroa control. Currently, mite control is practiced within an apiary rather than at an area-wide level. As shown in a study by Frey and Rosenkrantz (2014), foragers with mites can enter colonies from infested hives up to 1.5 km away. Therefore, mite populations should be monitored frequently especially in the fall when they can increase rapidly and infest colonies as they go into winter. Multiple fall treatments might be needed to maintain low varroa levels if hives are reinfested with mites from nearby apiaries. However, the results from our longitudinal study where colonies received four miticide treatments including two in the fall and still had unsustainable losses demonstrate the severe limitations of controlling varroa with miticides alone. Colony management practices also should be included to control varroa. For example, putting hives in cold storage facilities in the fall would reduce miticide applications by taking colonies out of environments where they could become reinfested. There is evidence that colonies stored indoors over the winter can have higher survival than those wintered outdoors (Bahreini and Currie 2015). Studies are needed to determine how best to prepare

colonies for cold storage, and to define storage conditions that optimize colony survival.

The varroa mite that entered the United States in the 1990s and was kept at low levels in managed colonies with well-timed miticide treatments perhaps no longer exists. The mites and the viruses they transmit have become increasingly harmful to bees, and the collapse of colonies has been co-opted as a dispersal mechanism. However, this cycle of mite increase, colony weakening, and mite dispersal can be broken by adopting beekeeping practices and mite control strategies with broader sensibilities to the type of pest that varroa has become.

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References Cited

Bahreini, R., and R. W. Currie. 2015. Influence of honey bee genotype and wintering method on wintering performance of *Varroa destructor* (Parasitiformes: Varroidae)-Infected honey bee (Hymenoptera: Apidae) colonies in northern climate. J. Econ Entomol. 108: 1495–1505.

Bailey, L., and B. Ball. 1991. Honey bee pathology. Academic Press, London, United Kingdom.

Carmo-Sousa, M., A. Moreno, M. Plaza, E. Garzo, and A. Fereres. 2016. Cucurbit aphid-borne yellows virus (CABYV) modifies the alighting, settling and probing behavior of its vector *Aphis gossypii* favoring its own spread. Ann. Appl. Biol. 169: 284–297.

Carrillo-Tripp, J., B. C. Bonning, and W. A. Miller. 2015. Challenges associated with research on RNA viruses of insects. Curr. Opin. Insect Sci. 8: 62–68.

Cervo, R., C. Bruschini, F. Cappa, S. Meconcelli, G. Pieraccini, D. Pradella, and S. Turillazzi. 2014. High Varroa mite abundance influences chemical profiles of worker bees and mite-host preferences. J. Exp. Biol. 217: 2998–3001.

Chen, Y. P., and R. Siede. 2007. Honey bee viruses. Adv. Virus Res. 70: 33–80.

Chen, Y. P., S. Pettis, A. Collins, and M. F. Feldlaufer. 2006. Prevalence and transmission of honey bee viruses. Appl. Environ. Microbiol. 72: 606–611.

Combes, C. 2005. The art of being a parasite, pp. 280. University of Chicago Press, Chicago, IL.

DeGrandi-Hoffman, G., and R. Curry. 2004. A mathematical model of Varroa mite (*Varroa destructor* Anderson and Trueman) and honeybee (*Apis mellifera* L.) population dynamics. Int. J. Acarol. 30: 259–274.

DeGrandi-Hoffman, G., and Y. Chen. 2015. Nutrition, immunity and viral infections in honey bees. Curr. Opin. Insect Sci. 10: 170–176.

DeGrandi-Hoffman, G., S. A. Roth, G. L. Loper, and E. H. Erickson Jr. 1989. BEEPOP: A honeybee population dynamics simulation model. Ecol. Modell. 45: 133–150.

DeGrandi-Hoffman, G., Y. Chen, E. Huang, and M. H. Huang. 2010. The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.). J. Insect Physiol. 56: 1184–1191.

DeGrandi-Hoffman, G., F. Ahumada, G. Probasco, and L. Schantz. 2012. The effects of beta acids from hops (*Humulus lupulus*) on mortality of *Varroa destructor* (Acari: Varroidae). Exp. Appl. Acarol. 58: 407–421.

DeGrandi-Hoffman, G., F. Ahumada, R. Curry, G. Probasco, and L. Schantz. 2014. Population growth of *Varroa destructor* (Acari: Varroidae) in commercial honey bee colonies treated with beta plant acids. Exp. Appl. Acarol. 64: 171–186.

DeGrandi-Hoffman, G., F. Ahumada, V. Zazueta, M. Chambers, G. Hidalgo, and E. W. DeJong. 2016. Population growth of *Varroa destructor* (Acari: Varroidae) in honey bee colonies is affected by the number of foragers with mites. Exp. Appl. Acarol. 69: 21–34.

De Jong, D., P. H. De Jong, and L. S. Goncalves. 1982. Weight loss and other damage to developing worker honeybees from infestation with *V. jacobsoni*. J. Apic. Res. 21: 165–167.

Delaplane, K. S., A. Berry, J. A. Skinner, J. P. Parkman, and W. M. Hood. 2005. Integrated pest management against *Varroa destructor* reduces colony mite levels and delays treatment threshold. J. Apic. Res. 44: 157–162.

Delaplane, K. S., J. van der Steen, and E. Guzman-Novoa. 2013. Standard methods for estimating strength parameters of Apis mellifera colonies. In V. Dietemann, J. D. Ellis, P. Neumann (eds.), The Coloss Beebook, Volume I: Standard methods for Apis mellifera research. J. Apic. Res. 52: http://dx.doi.org/10.3896/IBRA.1.52.1.03

Del Piccolo, F., F. Nazzi, G. Della Vedova, and N. Milani. 2010. Selection of *Apis mellifera* workers by the parasitic mite *Varroa destructor* using host cuticular hydrocarbons. Parasitology 137: 967–973.

DeMiranda, J. R., and I. Fries. 2008. Venereal and vertical transmission of deformed wing virus in honeybees (*Apis mellifera* L.). J. Invertebr. Pathol. 98: 184–189.

DeMiranda, J. R., G. Cordoni, and G. Budge. 2010. The Acute bee paralysis virus-Kashmir bee virus-Israeli acute paralysis virus complex. J. Invertebr. Pathol. 103.

DiPrisco, G., D. Annoscia, M. Margiotta, R. Ferrara, P. Varricchio, V. Zanni, E. Caprio, F. Nazzi, and F. Pennacchio. 2016. A mutualistic symbiosis between a parasitic mite and a pathogenic virus undermines honey bee immunity and health. Proc. Natl. Acad. Sci. (http://www.pnas.org/content/113/12/3203)

Donzé, G., M. Herrmann, B. Bachofen, and P. M. Guerin. 1996. Effect of mating frequency and brood cell infestation rate on the reproductive success of the honeybee parasite *Varroa jacobsoni*. Ecol. Entomol. 21: 17–26.

Francis, R. M., L. Nielsen, and P. Kryger. 2013. Varroa-Virus interaction in collapsing honey bee colonies. PLoS ONE 8: e57540. doi:10.1371/journal.pone.0057540

Frey, E., and P. Rosenkrantz. 2014. Autumn invasion rates of *Varroa destructor* (Mesostigmata: Varroidae) into honey bee (Hymenoptera: Apidae) colonies and the resulting increase in mite populations. J. Econ. Entomol. 107: 508–515.

Frey, E., H. Schnel, and P. Rosenkranz. 2011. Invasion of *Varroa destructor* mites into mite-free honey bee colonies under the controlled conditions of a military training area. J. Apic. Res. 50: 138–144.

Fuchs, S. 1990. Preference for drone brood cells by *Varroa jacobsoni* Oud in colonies of *Apis mellifera* carnica. Apidologie 21: 193–199.

Genersch, E. 2010. Honey bee pathology: Current threats to honey bees and beekeeping. Appl. Microbiol. Biotechnol. 87: 87–97.

Genersch, E., and M. Aubert. 2010. Emerging and re-emerging viruses of the honey bee (*Apis mellifera* L.). Vet. Res. 41: 54–74.

Genersch, E., W. Von Der Ohe, H. Kaatz, A. Schroeder, C. Otten, R. Buchler, S. Berg, W. Ritter, W. Muehlen, S. Gisder, et al. 2010. The German bee monitoring project: A long term study to understand periodically high winter losses of honey bee colonies. Apidologie 41: 332–352.

Greatti, M., N. Milani, and F. Nazzi. 1992. Reinfestation of an acaricide-treated apiary by *Varroa jacobsoni* Oud. Exp. Appl. Acarol. 16: 279–286.

Grozinger, C., J. Richards, and H. R. Mattila. 2014. From molecules to societies; mechanisms regulating swarming behavior in honey bees (*Apis* spp.). Apidologie 45: 327–346.

Heil, M. 2016. Host manipulations by parasites: Cases, patterns, and remaining doubts. Front. Ecol. Evol. 4: 80. doi: 10.3389/fevo.2016.00080.

Hoover, K., M. Grove, M. Gardner, D. P. Hughes, J. McNeil, and J. Slavicek. 2011. A gene for an extended phenotype. Science 333: 1401.

Iqbal, J., and U. Mueller. 2007. Virus infection causes specific learning deficits in honeybee foragers. Proc. Biol. Sci. 274: 1517–1521.

Koch, W., and W. Ritter. 1991. Experimental examinations concerning the problem of deformed emerging bees after infestation with *Varroa jacobsoni*. Zentralbl. Veterinarmed. B. 38: 337–344.

Le Conte, Y., G. de Vaublanc, D. Crauser, F. Jeanne, J. C. Rousselle, and J. M. Bécard. 2007. Honey bee colonies that have survived *Varroa destructor*. Apidologie 38: 566–572.

Lemaitre, B., and J. Hoffmann. 2007. The host defense of *Drosophila melanogaster*. Annu. Rev. Immunol. 25: 697–743.

Loftus, J. C., L. Smith, and T. D. Seeley. 2016. How honey bee colonies survive in the wild: Testing the importance of small nests and frequent swarming. PLoS ONE 11: e0150362. doi:10.1371/journal.pone.0150362

Marcangeli, J., L. Monetti, and N. Fernandez. 1992. Malformations produced by *Varroa jacobsoni* on *Apis mellifera* in the province of Buenos Aires, Argentina. Apidologie 23: 399–402.

Martin, S. J. 1994. Ontogenesis of the mite *Varroa jacobsoni* Oud. in worker brood of the honeybee *Apis mellifera* L. under natural conditions. Exp. Appl. Acarol. 18: 87–100.

Martin, S. J. 1995. Ontogenesis of the mite *Varroa jacobsoni* Oud. in drone brood of the honeybee *Apis mellifera* L. under natural conditions. Exp. Appl. Acarol. 19: 199–210.

McMenamin, A. J., and E. Genersch. 2015. Honey bee colony losses and associated viruses. Curr. Opin. Insect Sci. 8: 121–129.

Michener, C. D. 1974. Colony multiplication, pp. 132–137. *In* C.D. Michener, The social behavior of the bees. Belknap Pres, Cambridge, MA.

Natsopoulou, M. E., P. McMahon, and R. J. Paxton. 2016. Parasites modulate within-colony activity and accelerate the temporal polyethism schedule of a social insect, the honey bee. Behav. Ecol. Sociobiol. 70: 1010–1031.

Nazzi, F., S. P. Brown, D. Annoscia, F. Del Piccolo, G. Di Prisco, P. Varricchio, G. Della Vedova, F. Cattonaro, E. Caprio, and F. Pennacchio. 2012. Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. PLoS Pathog. 8: e1002735. doi:10.1371/journal.ppat.1002735.

Ostiguy, N., and D. Sammataro. 2000. A simplified technique for counting *Varroa jacobsoni* Oud. On sticky boards. Apidologie 31: 707–716.

Pedgley, D. E. 1993. Managing migratory insect pests-A review. Int. J. Pest Manag. 39: 3–12.

Peng, Y. S., Fang, S. Xu, and L. Ge. 1987. The resistance mechanism of the Asian honey bee, *Apis cerana* Fabr., to an ectoparasitic mite, *Varroa jacobsoni* Oudemans. J. Invertebr. Pathol. 49: 54–60.

Rosenkranz, P., P. Aumeier, and B. Ziegelmann. 2010. Biology and control of *Varroa destructor*. J. Invertebr. Pathol. 103: S96–S119.

Runckel, C., M. L. Flenniken, J. C. Engel, J. G. Ruby, D. Ganem, R. Andino, and J. L. DeRisi. 2011. Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, *Nosema*, and *Crithidia*. PLoS ONE 6: e20656. doi:10.1371/journal.pone.0020656.

Sakofski, F., N. Koeniger, and S. Fuchs. 1990. Seasonality of honey bee colony invasion by *Varroa jacobsoni* Oud. Apidologie 21: 547–550.

Seeley, T. D. 2007. Honey bees of the Arnot Forest: A population of feral colonies persisting with *Varroa destructor* in the northeastern United States. Apidologie 38: 19–29.

Seeley, T. D., and M. L. Smith. 2015. Crowding honeybee colonies in apiaries can increase their vulnerability to the deadly ectoparasite *Varroa destructor*. Apidologie 716–727.

Seitz, N., K. S. Traynor, N. Steinhauer, K. Rennich, M. E. Wilson, J. D. Ellis, R. Rose, D. R. Tarpy, R. R. Sagili, D. M. Caron, et al. 2015. A national survey of managed honey bee 2014–2015 annual colony losses in the USA. J. Apicul. Res. 54: 292–304. DOI:10.1080/00218839.2016.1153294

Shah, K. S., C. Evans, and M. C. Pizzorno. 2009. Localization of deformed wing virus (DWV) in the brains of the honeybee, *Apis mellifera* Linnaeus. Virol. J. 6: 182.

Simpson, J., and I.B.M. Ridel. 1963. The factor that causes swarming in honeybee colonies in small hives. J. Apic Res. 2: 50–54.

Toth, A. L., and G. E. Robinson. 2005. Worker nutrition and division of labor in honeybees. Anim. Behav. 69: 427–435.

Ushitani, T., C. J. Perry, K. Cheng, and A. B. Barron. 2016. Accelerated behavioural development changes fine-scale search behavior and spatial memory in honey bees (*Apis mellifera* L.). J. Exp. Biol. 219: 412–418.

Winston, M. L. 1987. The biology of the honey bee. Harvard University Press, Cambridge, England.

Yang, X., and D. Cox-Foster. 2007. Effects of parasitization by *Varroa destructor* on survivorship and physiological traits of *Apis mellifera* in correlation with viral incidence and microbial challenge. Parasitology 134: 405–412.

Yue, C., M. Schröder, S. Gisder, and E. Genersch. 2007. Vertical-transmission routes for deformed wing virus of honeybees (*Apis mellifera*). J. Gen. Virol. 88: 2329–2336.