Global Status of Honey Bee Mites

CHAPTER

5

Diana Sammataro

Introduction

Mites have numerous unique roles in the world's ecosystems and their incredibly rich body forms complement many amazing life histories. These invertebrates are in the Superorder Acari (from Greek for mite: *akari*), which include animals as small as 100 μm and as big as 10 mm (Red Velvet Mites). Mite fossils are found in the Devonian, around 400 million years ago, making them one of the earliest terrestrial lifeforms. Acarologists propose there are around 50,000 named species of mites, but suggest that number is a small fraction of those estimated to be discovered, identified, and named. This is an exceptionally large number of organisms about which we know very little; acarines are usually unnoticed or overlooked even by other scientists, mostly because of their limited economic importance and their small size.

Mites utilize any environment in which animals or plants are present and may equal or even surpass insects in the diversity of habitats and life cycles. Colonization includes soil, water (fresh and salt), in and on plants, arthropods, vertebrates, and invertebrates. Mites are found in polar extremes and desert environments and are even found 33 feet (10 m) deep in the ground (Walter et al., 1996). Some unexpected habitats include, for example, monkey lungs (Leonovich, 2010), sea snake tracheae (Nadchatram, 2006), special pouches on female carpenter bees (Scaife, 1952), and moth ears (Treat, 1957). It is only when mites cause injuries or affect the health of plants and animals (including humans) that they attract the interest of scientists. Mites that have received worldwide attention for these reasons include (but are not limited to) the spider mites (family Tetranychidae), the mange mites (family Sarcoptidae), and the allergen-producing house dust mites (family Pyroglyphidae). The tick vectors (ticks are large mites in the family Ixodidae) of Lyme disease are very well studied because of their deleterious effects on humans and other mammals (Steere, 2001); and hair follicle mites (family Demodecidae) have even been used in forensics (Desch, 2009).

Abstract Parasitic bee mites have become a major problem for both beekeepers and honey bees. This chapter updates the latest information on the three parasitic bee mite genera (*Acarapis*, *Varroa*, and *Tropilaelaps*) as well as newly identified species that are currently infesting bee colonies throughout the world. Monitoring and treatment options are discussed as well as mite behavior and future research directions.

Table 1. Mesostigmatic mites parasitizing honey bees, arranged according to host bee species.
(Compiled by D. Sammataro and D. L. Anderson; from Anderson and Morgan, 2007 and Navajas et al., 2010.)

Bee Host	Mite Species				
	<i>V. destructor</i>	<i>V. jacobsoni</i>	<i>V. underwoodi</i>	<i>V. rindereri</i>	<i>Euvarroa sinha</i>
<i>Apis florea</i>			X Nepal, S. Korea		X
<i>A. andreniformis</i>					
<i>A. cerana</i>	X	X	X		
<i>A. koschenikovi</i>				X Sumatra	
<i>A. nuluensis, Borneo</i>		X	X ?		
<i>A. nigrocincta, Sulawesi</i>		X ?	X		
<i>A. dorsata dorsata</i> Asia, Indonesia, Palawan	## Korea				
<i>A. d. breviligula</i>					
<i>A. d. binghami</i> Sulawesi					
<i>A. laboriosa</i> Nepal					
<i>A. mellifera</i>	X Japan & Korean haplotypes	X Papua New Guinea, Irian jaya	##		
<i>A. m. scutellata</i> Africa	X				

X —Positive identification, ** — Currently unresolved; ## — Incidental visitor.

Mites are also one of the largest and most diverse of bee associates. And eusocial bees especially have many mites; most of these mites benefit from the conditions of the hive environment, that is, it is protected, thermoregulated, and contains an abundance of year-round food. Over 30 mite species associated with honey bees (*Apis* spp.) are listed by Eickwort (1988), including those that are predatory, incidental, facultative, obligatory, or phoretic. The three major suborders of the mites on this list were the Astigmata (mostly detritus feeders), Prostigmata (mainly pollen feeders), and Mesostigmata (feeding on hive products such as bee bread as well as on bees) (Eickwort, 1988). Also on the list were three obligate parasitic mites; they are the Varroa mite (*Varroa* spp.), the tracheal mite (*Acarapis woodi*), and *Tropilaelaps* spp. (the latter is not yet reported in the Americas). The global spread of these parasites has had a profound impact on bees and beekeepers, due to massive losses and increased mite-associated pathologies (mostly bee viruses).

Bee mites, particularly Varroa mites, are now a major topic in scientific articles; over 1,700 journal articles on Varroa have been published since 1971, 44 in 2010 alone, compared to 102 articles on tracheal mites since 1934. *Tropilaelaps* mites appear in 28 articles and *Euvarroa* in seven.

Table 1. (continued)

	Mite Species				
Bee Host	<i>E. wongsirii</i>	<i>Tropilaelaps clareae</i>	<i>T. koengerum</i>	<i>T. mercedesae</i> n.sp.	<i>T. thaii</i> n.sp.
<i>Apis florea</i>					
<i>A. andreniformis</i>	X				
<i>A. cerana</i>					
<i>A. koschenikovi</i>					
<i>A. nuluensis</i> , Borneo					
<i>A. nigrocincta</i> , Sulawesi					
<i>A. dorsata dorsata</i> Asia, Indonesia, Palawan			X Sri Lanka	X Palawan, Sri Lanka	
<i>A. d. breviligula</i>		X Philippines (not Palawan)			
<i>A. d. binghami</i> Sulawesi				X**	
<i>A. laboriosa</i> Nepal			X	X Vietnam	X Vietnam
<i>A. mellifera</i>		X Philippines		X	
<i>A. m. scutellata</i> Africa					

X — Positive identification; ** — Currently unresolved; ## — Incidental visitor.

Tracheal Mite (Prostigmata: Tarsonemidae)

The first report of problems came when bees dying in the Isle of Wight between 1904 and 1919 were examined more closely. In 1921 the tracheal mite *Acarapis woodi* (Rennie) was identified in the breathing tubes or tracheae of bees where it feeds and reproduces (see Figure 1) (Eckert, 1961; Delfinado-Baker, 1982). Its detection led to the restriction of all live honey bee imports into the United States in 1922 (Phillips, 1923). Despite this precaution, the New World eventually became infested, perhaps via African bees that were transported to South America in the 1970s. By the early 1980s, this mite made its way to Mexico. The first report attributing mites to problems with bees came from beekeepers in Texas in 1984. Thereafter, *Acarapis* spread to all of the states, facilitated by commercial beekeepers transporting bees for pollination, and from the sale of mite-infected package bees from the southern states. In addition, infected swarms and drifting bees also contributed to the spread of the mite. *Acarapis* mites are probably distributed worldwide, wherever the Western honey bee (*A. mellifera*) has been imported. There are two external

Figure 1. Top, scanning electron micrograph of tracheal mite. Bottom, oval shadows in tracheal tube (left) are mites, a clean tube is on the right. $\times 400$. (Photo A by W. Styer; photo B by D. Sammataro.)

species in the genus *Acarapis* (*A. externus* and *A. dorsalis*), which are specific to the western honey bee (de Guzman et al., 2001). Currently, there are no reports of *Acarapis* species on other *Apis* bees.

Initially, *A. woodi* caused devastating losses throughout North America (see Figure 1, and 2). (For a complete life history, see Sammataro et al., 2000; Pettis, 2001; Wilson et al., 1997.) Tracheal mites interfere with the overwintering capability of colonies (bees are unable to form and maintain temperatures in the winter cluster) and are associated with paralyzed bees displaying disjointed wings (or K-wing) and crawling on the ground near hives. The actual cause for these early colony losses is still not clearly understood. Because of its small size, dissection of the honey bee is the only reliable way to determine infestation (Sammataro, 2006; and see video of bee dissection at: <http://www.ars.usda.gov/pandp/docs.htm?docid=14370>).

Control measures were vigorously studied and included vapors from menthol crystals, chemical acaricides, and oil or grease patties made from vegetable shortening and sugar (Sammataro et al., 1994; 2000). Breeding queens for resistance was another successful control method (de Guzman et al., 1998; Villa, 2006) and probably would have solved the problem. However, this endoparasite was soon overshadowed by the introduction of a second, more serious ectoparasitic mite, *Varroa*. However, *Acarapis* is still found but not readily seen because beekeepers are using multiple controls for *Varroa*.

Figure 2. Chart of the life cycle of tracheal mites. (Illustration by Signe Nordin.)

Figure 3. Differences in appearance between Varroa, Euvarroa and Tropilaelaps. Bar is 1mm.

(Illustrations by G.W. Otis and J. Kralj.)

Euvarroa (Mesostigmata: Varroidae)

The ectoparasite *Euvarroa* was first identified in 1974 from *Apis florea*, the dwarf honey bee from India (Aggarwal and Kapil, 1988) and is reported to only parasitize drone brood. These mites are smaller than Varroa and currently there are two known species: *E. sinhai* on *A. florea* throughout its natural range, and *E. wongsirii* on *A. adeniformis* from Malaysia and Thailand (see Table 1 and Figure 3). *E. sinhai* has 34 to 46 long setae or hairs on the posterior edge of the pear-shaped body shield; *E. wongsirii* has more hairs and is more triangular in overall body shape (Otis and Kralj, 2001).

Their biology is not well known other than they seem to infest only drone larvae of *A. florea* and have little impact on *A. mellifera*. They are found on the comb and will feed on adult workers, though they seem to prefer adult drones. The seasonality of drone brood production is probably the significant limiting biological factor for *Euvarroa* reproduction. While seemingly an important pest, bees appear to abscond when mite populations become elevated. Grooming behavior to remove the mite has not been observed in *A. florea*. While *Euvarroa* has been collected from *A. mellifera* and *A. cerana* colonies, they appear unable to reproduce in them (D. Anderson, personal communication); for a complete life history, see Otis and Kralj (2001).

Varroa (Mesostigmata: Varroidae)

The Varroa mite was first identified in 1904 as *Varroa jacobsoni* (Oudemans) from specimens found on the Asian honey bee, *A. cerana* in Indonesia, where it reproduced only in drone brood and otherwise caused little damage to the bee

Figure 4. Chart of the life cycle of tracheal and Varroa mites. (Illustration by Signe Nordin.)

colony. Since then, two other species have been described: *V. underwoodi* (Delfiando-Baker and Aggarwal, 1987) and *V. rindereri* (de Guzman and Delfinado-Baker, 1996; de Guzman et al., 1999; Warrit et al., 2006; Otis and Kralj, 2001); see Figure 3. Besides being morphologically distinct from *V. jacobsoni*, these two mite species are found on most Asian bee species (see Table 1). But once the European, or Western honey bee (*A. mellifera*) became established in Asia, *V. jacobsoni* was found to reproduce on both drone and worker brood, causing significant colony losses. In the 1980s, differences in *V. jacobsoni* were noted from diverse regions. Variations in the shape and size of adult females, in their reproductive biology, and in the parasite-host interface (Warrit et al., 2006; Rosenkranz et al., 2010) were recorded. Meanwhile, *A. mellifera* colonies in Europe, North America, and the Middle East were quickly succumbing to Varroa infestation, yet honey bees in the tropics of South America were not affected. Ultimately, molecular techniques revealed that Varroa mites from Asia were genetically distinct from those in the United States. In 2000, *V. jacobsoni* infesting the Western honey bee was found to be yet another species and was renamed *V. destructor* (Anderson and Trueman, 2000). After further study, 18 different haplotypes (mites with unique mitochondrial [mtDNA] sequences) have been recorded: nine in *V. jacobsoni*, six in *V. destructor*, and three that are still unresolved at this writing (Navajas et al., 2010).

The haplotypes are named after the country where the mite was first found. Two of the *V. destructor* haplotypes from the Asian honey bee, J1 and K1 (Japan 1 and Korea 1), have successfully adapted to *A. mellifera*. J1 shifted onto *A. mellifera* in Japan, Thailand, and Brazil, and finally into North America. K1 transferred to *A. mellifera* near Vladivostok, Russia, then to Europe and North America by 1987 (Navajas et al., 2010). More genetic variations of Varroa were also discovered off their native host *A. cerana*, but the K1 and J1 groups on the Western honey bee show little genetic diversity even though they are now widespread on *A. mellifera* in

Figure 5. Comparative sizes. **A.** *Acarapis* (arrow). **B.** Flea. **C.** A female *Varroa*.
(Photos A–B by W. Styer; photo C by C. Pooley.)

Asia. Currently there are at least two new haplotypes (J1-5 and K1-2) in Asia on *A. mellifera* (Navajas, et al., 2010).

The shift of these mites to the Western honey bee was facilitated by beekeepers introducing this susceptible species into Asia as far back as the 1950s. The accelerated dispersal of this relatively large, visible ectoparasite in North America was aided by colony movement for pollination or honey production (see also <http://www.mylovedone.com/image/solstice/win10/SammataroandArlinghaus>).

Physically, female *Varroa* are 1.1 mm long by 1.6 mm wide, weighing between 0.2 to 0.5 mg (live, fresh weight), depending on whether it is actively laying eggs or not (Sammataro and Finley, unpubl. data). Detailed information on the *Varroa* life cycle is found elsewhere (Sammataro et al., 2000; Martin, 2001; Rosenkranz et al., 2010); see Figure 3, 4, and 5. In general, female *Varroa* infest the 5th instar bee larvae just prior to capping (Day 7–8 of larval life). Drone larvae are preferred but workers are also parasitized. Once in the cell for about 70 hours, the female lays a male egg (Rosenkranz et al., 2010), followed by female eggs every 30 hours. Up to five eggs (average three) can be laid in worker larvae; but because of their longer development time, the mother mite can lay up to six eggs on drone larvae (which is why drone larvae are preferred).

Mites are able to find the correct-aged brood cells by means of chemical cues released by the bee larvae (Rickli et al., 1992; Rosenkranz et al., 2010). They will feed on larval hemolymph while going through their own developmental stages of proto- and deutonymphs, followed by the adult mites. This feeding weakens the bees, reducing hemolymph, transferring virus, and modifying their volatile profile (Salvy et al., 2001). Male mites are much smaller and once they mate with their sisters, die in their natal cell. The mature, mated females (daughters and the mother) will then emerge with the teneral bee and search for new larvae with which to begin the cycle anew. If no brood is present, as in winter months, mites can cling to adult bees, feeding intermittently on bee hemolymph until brood is once again available. Richards et al. (2011) found that the saliva from *Varroa* mites facilitates their ability to feed by suppressing the wound-healing capabilities of the bees. Further research on mite behavior and reproduction is sorely needed if new control tactics are to be developed and adaptive characteristics of *Varroa* on *A. mellifera* are to be understood.

Tropilaelaps (Mesostigmata: Laelapidae)

Tropilaelaps is the newest threat to global apiculture (Baker, 2010), but for now is confined to its Asian home range. Four species have now been described and, according to Anderson and Morgan (2007), the two species, *Tropilaelaps clareae* and *T. koenigerum*, are found on the giant honey bees of Asia, *Apis dorsata dorsata* and *A. laboriosa* (see Table 1 and Figure 3). *T. clareae*, an important pest of the introduced Western honey bee (*A. mellifera*) is found throughout Asia. However, *T. clareae* is now considered to be two species, consisting of haplotypes parasitizing the native *A. d. breviligula* and the introduced *A. mellifera* in the Philippines (except Palawan) and on Sulawesi Island in Indonesia, parasitizing the native *A. d. binghami* (Anderson and Morgan, 2007). The new *T. mercedesae* n. sp., (previously mistaken for *T. clareae*) and common in Sri Lanka, now includes haplotypes that parasitize the native *A. d. dorsata*, *A. laboriosa*, and the introduced *A. mellifera*. *T. koenigerum* is found on *A. d. dorsata* and *A. laboriosa*. Another new species, *T. thaii* n. sp., has been recently discovered on *A. laboriosa*; for complete details, see Anderson and Morgan (2007).

Tropilaelaps mites are around 1 mm in length, red-brown in color, and are elongated compared to the more oval Varroa (Figure 3). They hold their first pair of legs like antennae and quickly move on the comb surfaces. These mites are found on the combs or on adult bees and prefer drone brood (but also are found on worker brood) in which to lay eggs. *T. clareae* cannot feed on adult bees, nor can they survive more than a few days as phoretic passengers, which is why they thrive in tropical climates. In temperate climates there is a long broodless period when the bees are in their winter cluster. During this time, because Tropilaelaps cannot feed on adults, they may not survive the long winter. *T. koenigerum* is slightly smaller, and reported to be harmless to *A. mellifera*, as is *T. thaii* (Anderson, CSIRO personal communication).

The geographic range of this mite has expanded since it adapted to *A. mellifera* (Anderson and Morgan, 2007; see Table 1). Tropilaelaps are considered an emerging global threat to beekeeping, not only for their injury to bees but because they also carry the deadly Deformed Wing Virus (DWV) (Forsgren et al., 2009; Dainat et al., 2009). The United States Department of Agriculture is conducting a survey (2010–11) to search for this mite, as well as for *Apis cerana*, that may have come in with shipments of package bees from Australia. Currently the USDA has banned all imports of live bees from Australia into the United States. This threat makes it imperative to update the quarantine protocols for all countries that import and export live honey bees.

Mites and Virus

The biggest problem with bee mites is that they not only damage individual bees by the feeding activity and reduce their lifespan and learning capabilities (see Rosenkranz et al., 2010), but they also carry pathogenic RNA viruses. Virus particles have been found in Varroa's salivary glands (Cicero and Sammataro, 2010) as well as in other parts of their body (Zhang et al., 2007). To date, 18 different viruses have been identified from bees and many of them are vectored by mites, especially, but not limited to Varroa (Chen and Siede, 2007; Forsgren et al., 2009; Dainat et al., 2009; Boncristiani et al., 2009). These viruses include Kashmir (KBV),

Sacbrood (SBV), Israeli Acute Paralysis (IAPV), and DWV (Santillán-Galicia et al., 2010; Highfield et al., 2009; de Miranda et al., 2010; de Miranda and Genersch, 2010; de Miranda et al., this edition). Much of the blame for the incidence of Colony Collapse Disorder (CCD) in 2006 in which many bee colonies died in the United States was attributed to Varroa-vectored viruses (Berthoud et al., 2010; Ellis et al., 2010; Boecking and Genersch, 2008; vanEnglesdorp et al., 2009; Schäfer et al., 2010).

Monitoring Mites

When symptoms of mite infestation appear in bee colonies (mites seen on bees and larvae, bees with deformed wings crawling on the ground, bees pulling out diseased brood, colony collapse), there are several methods to determine what kind of ectoparasitic mites are present and to estimate their numbers. The optimal time to monitor mite levels is before they become a problem; twice per year (spring and fall) is often recommended. If mite populations are growing, monitoring colonies every two months may be necessary. Sampling 10% of colonies in an apiary is often mentioned, but more recent work by Lee et al. (2010a,b) showed that eight colonies per apiary gave enough information on Varroa mite densities.

Monitoring mites is useful because if mite populations are low, no treatments are needed and beekeepers can save money (on treatments) and time. Fewer treatments will also help diminish the buildup of resistance to acaricides, thus reducing the amount of product needed. Also, the reduced use of pesticides in the colony lessens contamination in the hive and its products. Collapsing colonies or those with high mite populations should be treated first and subsequently monitored, which will indicate whether control methods have been effective. The procedures listed below can help in estimating the number of mites in a particular colony and augment treatment times or options. They are broken into two broad categories, *active* (or invasive) and *passive* sampling.

Active Sampling for Mites

Ether Roll

Pick a colony and brush or shake 300 bees from one to three randomly selected frames containing brood, as mites usually are on the nurse bees that are on brood frames (Lee et al., 2010b). Bees should be brushed into a wide-mouth jar or shaken from frames into a larger container first, until 300 bees are collected. Queens should not be sampled. Knock the bees to the bottom of the jar with a sharp blow and measure a 2-inch (5.08 cm) line with a grease pencil or permanent marker to make this step more accurate. Note: A 1/3 cup (78.07 mL) of bees is between 238 to 300 bees; 1/4 cup (59.15 mL) is about 200 bees; a 100-mL beaker will hold 300 bees. Spray a 2-second burst of ether auto starter fluid and cover the jar with the lid. When the bees die, they will regurgitate the contents of the honey stomach onto the sides of the jar, making it sticky. Shake the bees vigorously for 1 to 2 minutes, then roll the jar horizontally to spread out the bees; the mites should stick to the sides of the jar. Count both mites and bees to determine the number of mites per 100 bees. CAUTION! Ether is HIGHLY flammable and should be used with vigilance near a lit smoker.

Figure 6. **A.** Collect bees by brushing them into a 100-mL beaker. **B.** Once the bees are transferred to a jar with a screen top, add powdered sugar; there should be 300 adult bees for a sugar shake. **C.** Shake the jar vigorously to dislodge the mites; shake over a paper towel or other white paper. **D.** Mites can be more easily seen when they fall on wet paper towel; insert: the mites are clearly visible. (Photos by D. Sammataro.)

The ether in this method can be replaced by 70% alcohol or soapy water (the latter is not a fire hazard); pour enough liquid into the jar to cover the bees (2 oz or 50 mL) and agitate the jar vigorously to dislodge mites. Strain out the liquid into another container, using a sieve or mesh to catch the bees; the mites will be in the liquid. Refill the jar with more liquid and shake again, repeating this at least 4 times to knock off all the mites. Filter the liquid and mites through a coffee or cloth filter to separate and count the mites and bees.

Sugar Shake

Instead of killing the bees, add 1 oz or 2 tablespoons (25 g) powdered or icing sugar (or flour) to the collecting jar with the 300 bees, and wait for about 2 minutes (Figure 6). Then put screening material (such as 8 × 8 mesh hardware cloth) on top of the jar and shake vigorously. The mites are best seen when shaken onto white paper to count; shake again and repeat at 2-minute intervals.

If an accurate mite per 100 bee count is desired, finish the sugar shake with an alcohol or soapy water wash to collect all the mites and count the bees in the samples (see Lee et al., 2010a,b). The number of mites in a colony can be estimated by doubling the number of mites/100 bees (Lee et al., 2010a,b; Fuchs, 1985); doubling the number of mites/100 bees accounts for the mites that are in the brood. Alternatively, after collecting and counting mites, use the chart (Table 2) to convert the number of counted mites to the percent of mites in a colony (colony infestation).

Colony and Brood Examination

Another method to monitor bee colonies for the presence of Varroa (or Tropilaelaps) is to examine both capped drone and worker brood. This can be done using a cappings scratcher (with fork-like tines) to pull up

# Mites per 300 Adult Bees	Colony Infestation	# Mites per 8 300-Adult-Bee Samples	Apiary Infestation
1	1 %	8	1 %
2	1 %	16	1 %
3	2 %	24	2 %
4	3 %	32	3 %
5	3 %	40	3 %
6	4 %	48	4 %
7	5 %	56	5 %
8	5 %	64	5 %
9	6 %	72	6 %
10	7 %	80	7 %
11	7 %	88	7 %
12	8 %	96	8 %
13	9 %	104	9 %
14	9 %	112	9 %
15	10 %	120	10 %
16	11 %	128	11 %
17	11 %	136	11 %
18	12 %	144	12 %

Table 2. Mite numbers per 300 adult-bee samples and the corresponding density in the colony; also the mites in eight 300-bee samples by apiary and the corresponding density in the apiary. After Lee et al. (2010b) and by permission of the author. This table is used instead of having to calculate mites per 100 bees.

Figure 7. A. Pulling up drone brood (note the bullet-like capped cells). B. Varroa mites on a drone larva. (Photos by D. Sammataro.)

capped pupae. Examining drone brood is preferred because the mites favor drones (longer development time) and drone brood is larger and easier to pull up; the loss of some drones has little if any impact on hive health (Fuchs, 1990; Schulz, 1984). However, an equal number of drone and worker brood should be examined for a more realistic mite count and to determine if *Tropilaelaps* is present.

Sample between 100 and 200 (or as much as 2,000) capped purple-eyed pupae. Slide the fork horizontally along the comb surface to puncture as many capped cells as possible and then rotate it upward, along with the cappings, pulling up the brood from the cells (Figure 7). The mites will be clearly visible on the white pupae. Using a bright light source, examine the now-empty brood cells to determine if the white fecal

material from Varroa is present on the cell walls; immature mites should be also visible inside the empty cells. Younger mite stages will be lighter in color; older mites are dark brown. Check several patches of brood within the colony to get a more complete picture of the mite distribution.

Another method to collect capped brood is to cut off the cappings (de-cap) with a serrated or heated knife (such as an uncapping knife used for honey) and wash out the brood with a stream of warm water. Brood can be rinsed in a coarse sieve (2 to 3 mm) and mites collected in a lower, fine sieve or screen (1 mm mesh) covered with filter paper to make them clearly visible. However, immature mites, using this method, may be overlooked or washed away.

Estimates of the infestation rate can be done by opening a predetermined number of brood cells to calculate a percentage of capped brood containing live mites. The accuracy of this method is, however, uncertain. Charriere et al. (2003) found that the mite numbers varied from one to six times even within a week of sampling, and Devlin (2001) reports that the method was best done during the fall season but not in spring and summer. Because of this, threshold levels using brood pulls have not been developed. The combination of the ether rolls and brood pulls may yield the most accurate estimate of mite density (Branco et al., 2006).

Treatment is usually called for if 10% to 12% of the colonies in an apiary (or a colony) are infested in the fall (see Table 2); but this can vary by region, bee population, and time of year. Keeping records of mite levels in colonies is a good way to determine if chemical treatments will be beneficial. Beekeepers in adjacent apiaries could cooperate in treatment schemes to keep mite levels low in a particular area.

Figure 8. GL-IPM Varroa board used to count natural mite drop; see website references. (Photo by D. Sammataro.)

Passive Mite Sampling

Sticky Board or Hive Debris

A precise diagnosis of mite infestation can be made using a sticky board that is made from a sheet of heavy cardboard, poster board, or other white stiff paper or plastic or laminated paper smeared with a sticky coating. The board should be covered with a wire mesh (Figure 8), such as screening material. The outside edges of the mesh can be folded under to raise it off the board and stapled or taped onto the board. This prevents the bees from removing the dislodged mites and keeps bees off the sticky material. The sticky board can be coated with Vaseline or another substance (or use a sheet of sticky shelf paper).

Boards can also be purchased that are already coated with a sticky material (good for hot climates) and that are visually easier to use when counting mites (Great Lakes IPM; Ostiguy and Sammataro, 2000; see Figure 8). The entire board needs to fit the full length of the bottom board of a hive. The board is left in the colony for up to 3 days, after which the debris can be examined for mites. It is important to use the full-size board to estimate mite populations; anything less will not give an accurate count. To determine that only mites are counted, a magnifier can be used to distinguish mites from other debris.

Boards are perfect as a noninvasive method to determine mite loads in a colony and, more importantly, the change of mite density over time (Sammataro et. al., 2002). However, it is difficult to predict the number of mites in a colony with this technique and the significance of the number of mites counted needs to be correlated with treatment requirements; how many mites constitutes a treatable number varies.

Table 3. Chemical controls for Varroa mites; compiled from Rosenkranz et al., 2010 and <http://www.maf.govt.nz/biosecurity/pests-diseases/animals/varroa/guidelines/control-of-varroa-guide.pdf>.

Product Trade Name ®	Active Ingredient	Chemical Class
Apiguard	Thymol	Essential oil
Apilife VAR	Thymol, eucalyptol, menthol, camphor	Essential oils
Apistan **	Fluvalinate	Synthetic pyrethroid
Amitraz, Miticur, Api-warol (tablets)	Formamidine	Formetanate, methanimidamide
Apitol	Cymiazole	Iminophenyl thiazolidine derivative
Apivar **	Amitraz	Amadine
Bayvarol **	Flumethrin	Synthetic pyrethroid
Check-Mite+ **	Perizin, coumaphos	Organophosphate
Folbex	Bromopropylate	Chlorinated hydrocarbon
Sucrocide	Sucrose octanoate	Sugar esters
Hivestan	Fenpyroximate	Pyrazole (alkaloid)
Generic (e.g., Mite Away™)	Formic acid	Organic acid
Generic	Lactic acid	Organic acid
Generic	Oxalic acid	Organic acid

** No longer effective in some areas.

Delaplane et al. (2005) estimated a treatment threshold (on a 24-hour sticky board) of 71 to 224 mites in August in the southeastern states and 1 to 12 mites in the spring. In the northwestern states, these numbers were calculated as 12 mites in the spring and 23 in the fall; for ether rolls, the numbers were 3 mites in April, 14 in August, and 4 in October (Strange and Sheppard, 2001). Treatment thresholds in other regions need to be carefully researched.

Use of Chemotherapy to Control Mites

A list of some of the current chemical miticides (acaricides) used to control Varroa is outlined in Table 3; many of these may work for Tropilaelaps. Optional treatments to reduce Varroa levels are always being tested by beekeepers and researchers. Below are some methods that have been tried or are being developed; comments by the authors:

- Chlorfenvinphos (organophosphate), effective but residues may cause problems (Milani et al., 2009); Apiwarol tablets are burned, but are toxic to bee brood (Dzierzawski and Cybulski, 2010).
- Azadirachtin (Neem), needs more work (González-Gómez et al., 2006; Peng et al., 2000; Melathopoulos et al., 2000; Fassbinder et al., 2002).
- Plant-derived monoterpenoids, some report bee toxicity (Fassbinder et al., 2002; Ali et al., 2002); other essential oils are being tried (Gashout and Guzman-Novoa, 2009; Ruffinengo et al., 2006; Shaddel-Telli et al., 2008; Garcia-Fernandez et al., 2008; Sammataro et al., 2009) and other compounds (Emsen and Dodoglu, 2009).

- Food grade mineral oil, not effective (Elzen et al., 2004).
- Powdered sugar dust (not effective for control; Ellis et al., 2009a) but has potential (Fakhimzadeh, 2000) and is good for sampling, (Aliano and Ellis, 2005; Macedo et al., 2002).
- Screen wire bottom boards; not effective in keeping mite populations low (Ellis et al., 2001; Harbo and Harris, 2004) but does help some, especially when used with other methods (Delaplane et al., 2005).
- Smoke, burning different plant materials; reports of some success but may be harmful to bees (Çakmak et al., 2002; Çakmak et al., 2006; Eischen and Wilson, 1998; Elzen et al., 2001a,b; O'Meara, 2005; Romeh, 2009).
- Thermal treatments, such as heating frames in an “oven” (Rosenkranz et al., 2010; Tabor and Ambrose, 2001).
- Cell size modifications (Maggi et al., 2010a); small cell size does not work (Berry et al., 2010; Ellis et al., 2009b).
- Mite traps or killing with specialized frame, for example, Mite Zapper (Huang, 2001).
- Propolis (Daminai et al. 2010; Garedew et al., 2002).
- Antioxidants (Sammataro et al. 2010).

Problems with Chemotherapy

Resistance and Residue

Varroa mites developed resistance to the chemical acaricides within 10 to 15 years after they were first used. Since Varroa's introduction into the United States in 1987, the first report of resistance to fluvalinate surfaced by late 1994 (Milani 1994, 1995, 1999) followed swiftly in other areas (Elzen et al., 1998 and 1999a,b). By 1998, coumaphos (Elzen and Westervelt, 2002; Lodesani et al., 1995) and later amitraz (Elzen et al., 1999c) were added to the list of resistant chemicals. By 2000, it was suggested to rotate different chemicals to keep ahead of the resistant mites (Elzen et al., 2001c). Reports of resistance vary by country but it appears to be a major problem, particularly in some newly infested areas. Increased resistance is driving commercial beekeepers to experiment with chemicals and other compounds in an effort to find a treatment that is economical, effective, and easy to use. Development of the pesticide “strip” has been described by some as a great disservice to the bee industry. The rationale is that “ease of use” of the early strips raised the expectations for an “instant solution” for control of the resistant mites. Preparation of untested acaricide cocktails by beekeepers to combat growing Varroa resistance has been an ongoing problem for years. Many of these recipes are harmful if ineffective and this kind of “experimentation” is discouraged by scientists.

Another common misconception is in the area of overdosing, such as doubling or tripling the amounts recommended on the label, which is both illegal and dangerous. Some products may kill the bees outright; the LD₅₀ of the compounds should give a clue as to how toxic they are (LD₅₀ indicates the individual dose required to kill 50% of a known population of honey bees). It does not indicate long-term effects, only short-term toxicity; the smaller the number, the more toxic the substance.

The LD₅₀ (in µg/bee) of many pesticide compounds are now online at: <http://www.ipmcenters.org/Ecotox/DataAccess.cfm>.

Another area of concern is that some acaricides contaminate wax and other hive products. The first paper to discover that agrochemicals were found in honey was Ogata and Bevenue (1973). Now there are hundreds of journal articles on residues in bee colonies. The greatest residue risks are with acaricides, but other pesticides are also identified. Mullin et al. (2010) reported that 121 different pesticides and metabolites were found in hive products (see Chapter 14, this edition). Documenting what compounds and their breakdown products do to all bee life stages as well as the beneficial microbes living in bees and bee colonies is the subject of intense research (Johnson et al., 2010a,b) and how they may affect the overall health of bees (Le Conte et al., 2010).

Effect on Drones

Varroa has had a great impact on feral bees to the point that many “wild” honey bee colonies have now disappeared or are in low numbers. Varroosis has an additional negative effect on male fitness, decreasing flight duration and reducing the number of spermatozoa produced. Drone survival is also significantly reduced in Varroa-infested colonies (Rinderer et al., 1999) and the identification of Deformed Wing Virus (DWV) in the epithelial cells of the seminal vesicle of drones (could also be present in the semen) may have a negative effect on drone fertility (Fievet et al., 2006; Chapter 8, this edition) and could be passed on in the eggs the queens are laying. Reduced sperm counts and drone flight translate into less sperm available to queens, either because the drones are not able to take long mating flights or the spermatozoa are not as viable. Queens are therefore not well-mated and usually quickly superseded.

Other Control Measures: Biotechnical Controls and IPM

One result of Varroa's resistance to some chemicals is the search for different control tactics. Such measures can be used in an Integrated Pest Management (IPM) strategy that utilizes multiple tactics, as represented in Figure 9 (and see Chapter 6, this edition). One successful IPM strategy for *Tropilaelaps* is treating the mites during the phoretic, broodless periods because they cannot feed on adult bees and, more importantly, they cannot survive without access to brood (Calis et al., 1999; Wilkinson and Smith, 2002). Caging the queen while removing drone brood will also help control both *Tropilaelaps* and Varroa populations. Dusting with powders (such as icing or powdered sugar) can also suppress mite levels (Aliano and Ellis, 2005; Ellis et al., 2009b). Three other IPM techniques are outlined below. IPM strategies for Varroa were studied by Delaplane et al. (2005) and they found that screened bottom boards and mite-resistant queens show significant reduction, especially when used together. However, they also reported that these methods had a negative effect, such as reduced honey and pollen storage. In some instances, the more mite-resistant queens produced less brood. IPM methods are best for reducing chemical treatments and for keeping track of mite populations; new strategies need to be developed.

Figure 9. IPM tactics for mite control. (Adapted by D. Sammataro with a template for IPM controls from Penn State University. Used with permission.)

Open Bottom Boards or Open Mesh Floor

Mites cannot survive for very long without host contact. For this reason, mites that fall off bees or comb through wire mesh floors will generally perish. Overall, mite populations are reduced using open bottom boards and by limiting the buildup of debris on the hive floor, benefits can be realized, such as improving hive ventilation and discouraging other pests such as the wax moth from nesting in hive waste on bottom boards; wax moths successfully exploit bottom debris and can kill weakened colonies. Cold winters may preclude the use of open bottom boards, so another kind of bottom board with a cover for the screen should be used. In warmer climates, however, the open floor is an invitation to ant invasions unless other precautions are taken. Open floor bottoms can be an integral part of *Varroa* and *Tropilaelaps* monitoring and control. However, open bottom boards, with or without the sticky board, are not sufficient on their own to significantly reduce mite levels and work best when used in concert with other control methods (Ellis et al., 2001; Delaplane et al., 2005; Harbo and Harris, 2001, 2004).

Comb Trapping and Drone Brood Removal

Using sheets of drone foundation in the brood chamber or in a super frame encourages bees to draw out drone comb (Charriere et al., 2003; Rosenkranz et al., 2010). When the queen lays eggs in drone cells, the drone population can increase. Because drones are more attractive to the *Varroa* mite, once the brood is capped over, it can be removed and the contents disposed of (Wantuch and Tapy, 2009), for instance by freezing the comb. However, there is a risk of increasing mite populations if the beekeeper fails to remove the sealed comb.

Breeding for Varroa Tolerance

Breeding bees to be resistant or tolerant to mites has been ongoing since mites were first discovered infesting the Western bees. The natural hosts of Varroa (and *Tropilaelaps*) are able to keep the mite–host relationship in balance by several mechanisms; for Varroa these are:

1. “Entombing” drone brood that is multiply infested, by not helping weakened drones emerge, thus containing the mites (Rath, 1992); this activity is found only in the *Apis cerana* bees.
2. Grooming behavior, where mites are removed by other bees (Peng et al., 1987); but this is difficult to establish in European colonies (Aumeier, 2001; Büchler et al., 2010; Stanimirovic et al., 2010).
3. Hygienic behavior, where the workers detect and remove infested brood (Spivak, 1996; Spivak and Reuter, 1998); interrupts the reproductive cycle of the mite. This trait has been refined into a line of bees called Varroa Sensitive Hygiene (Harbo and Harris, 2001; Harris et al., 2010). In addition, bees from eastern Russia that were imported into the United States. (Rinderer et al., 2001, 2010; Çakmak, 2010) show some tolerance to Varroa.
4. Nonreproductive or reduced fecundity of mites, which appears mostly in African or Africanized bees (Rosenkranz, 1999), is apparently too variable to be a factor in reducing mite levels in Western honey bees (Rosenkranz et al., 2010).
5. Mites damaged by bees is currently being re-evaluated as a heritable trait in bees and could be used in a selective breeding program (T. Webster, personal communication); for more information, see Corrêa-Marques et al. (2000).

Some reports on the downside of breeding mite resistance in honey bees document reduced honey production, elevated defensive behavior, increased brood disease incidence, small bee populations over winter, and increased swarming tendencies. In general, beekeepers want thriving bee populations with gentle behavior and high honey yields, so natural selection has a long, slow road ahead. For more information see Fries (chapter 3, this edition) and Rosenkranz et al. (2010).

New Treatments and Future of Varroa Research

Currently, acaripathogenic fungi are being evaluated for Varroa control, including the testing of *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii*, and *Hirsutella thompsonii*. Two serious drawbacks to using fungi are that they take several days to infect and kill their target, and they may not be optimally adapted to the brood nest environment (Chandler et al., 2001; James et al., 2006; Kanga et al., 2002, 2003, 2006, 2010; Meikle et al., 2006, 2007, 2008a,b; Peng et al., 2002; Rodríguez et al., 2009a,b; Shaw et al., 2002; Steenberg et al., 2010).

Another new biological agent being tested is the bacterium *Serratia marcescens*, which was isolated from *A. cerana* bees; it secretes proteins that act as a biological degrader of chitin (Tu, 2010). While essential oils are efficacious, volatility issues make it problematic for primary mite control. Consequently, new delivery systems are being tested, including porous ceramics (Booppha et al., 2010) and encapsulating oils in starch (Glenn et al., 2010). RNA interference (or RNAi) and other molecular techniques may hold promise in controlling Varroa and even preventing

bee diseases (Campbell et al., 2010; Liu et al., 2010; Maori et al., 2009; Paldi et al., 2010; Schlüns and Crozier, 2007). The idea is to silence particular genes or regulate gene expression in microbial diseases and mites that would be fatal to those organisms.

The attractiveness of brood and the release of brood volatiles are still under investigation (Piccolo et al., 2010). Varroa attractant and arrestment responses to brood volatiles are currently being investigated for use in bait traps (Carroll, M. J., USDA, personal communication). New controls are always a top priority for Varroa, but more research is needed to investigate the effects pesticides have on the Varroa life cycle, and the interactions of contaminants now found within the hive environment. Other areas of investigation to combat these mites, especially Varroa, and possibly *Tropilaelaps*, will be in overcoming resistant mites. Acaricide resistance is now a reality in the United States and most likely a global issue. We must understand the changes in this mite from the Varroa that was present 20 years ago, and to study its life cycle more thoroughly. New controls need to be developed that will not contaminate the colony or pose resistance problems. One important area is rearing Varroa off-host, on an artificial diet. In this way we will be able to understand the complete life cycle of the mite, their diet requirements, reproductive strategies, and weaknesses. At a recent COLOSS workshop (<http://www.coloss.org/>) on “Varroa, Viruses and Standardization of Methods” held in Magglingen, Switzerland (November 2010), the following list of Varroa research priorities was recorded:

- Biological control (pheromones, entomopathogens, endosymbionts) and IPM
- Trigger of Varroa reproduction (in original and new host, including the geographic and genetic variation)
- Development of Varroa *in vitro* rearing and reproduction
- Search for Varroa-tolerant bees and identify the tolerance mechanisms for breeding programs and the problem of narrowing genetic diversity
- Host parasite co-evolution; local adaptations, *V. jacobsoni* and *V. destructor* on *Apis cerana*, role and maintenance of genetic diversity
- New invasive mites (*Varroa* spp. and *Tropilaelaps* spp.)
- *V. destructor* genome
- Modeling of population dynamics
- Mite virulence thresholds (including virus transmission and replication)
- Investigation of Varroa invasion on virus presence in populations
- Virus transmission and virulence

There is still a lot to learn and research areas are changing fast. New molecular tools may help in our understanding of these mites and eventually may assist us in learning how to mitigate the bad things these mites do to bees. Even at this writing, new breakthrough research may have already solved the bee-mite problem.