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Looking to Nature to Solve the Health Crisis of Honey Bees

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Figure 1.1 *Gathering honey*, a beekeeping scene from the Tomb of Rekhmire. Egypt c. 1450 BCE (de Garis Davies 1930).

Prologue

Scientists recently discovered the lipid residues of ancient beeswax inside the earthen pottery vessels of Neolithic farmers, which suggests that the origin of domestication of honey bees dates back to the onset of agriculture (Roffet-Salque et al. 2015). The long association between humans and bees (Figure 1.1), with mankind harnessing honey bees for food, medicine, and spiritual wellness, can be summed up in a single word: beekeeper. In this book, we introduce a new term to the English language: bee doctor. Etymologists, who study word metamorphosis, follow how the use of particular words gradually evolve in our language – e.g. from bee keeper, to bee-keeper, and finally to beekeeper. Just as the "honey bee" is spelled as two separate words because it is a true bee, we will likewise separate "bee" and "doctor" since bee veterinarians are true doctors in every sense of the word. We work from single bee to whole colony, from individual cell to multicellular organism, and from microenvironment to ecosystem. Given the urgent call for modern *Homo sapiens* to reverse the anthropogenic impacts on pollinators everywhere, including our sacred *Apis mellifera*, we propose adoption of "bee doctor" without delay. Humans have been "keeping" bees for thousands of years, so we now have the word "beekeeper." Only by forging a close connection between human beings and honey bees in all matters relating to their health, do we stand a chance to save one of earth's most industrious species – the one who gives us food, health, and happiness, and was idolized on the walls of Egyptian tombs. Perhaps someday we will even have the word "beedoctor."

A Tenet of Medicine: Learn the Normal

Colonies of honey bees living in the wild are prospering in American forests even in the face of myriad stressors that are decimating the managed colonies living in apiaries. We know that both cohorts are exposed to the same parasites and pathogens. How then do wild colonies survive without beekeeper inputs, whereas managed colonies live just one to two seasons if humans do not intervene with various supplements or medicines? In examining this conundrum, we must ask ourselves as bee doctors, working hand-in-hand with beekeepers, how we should examine the health of the honey bee? A fundamental tenet of medicine is the need to learn what is *normal* (regarding anatomy, physiology, or the state of being known as health) before one can

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understand deviations from this baseline. We contend that the "normal" that bee veterinarians should be concerned about is the wonderfully adapted lifestyle of wild colonies of honey bees. In this chapter, we will highlight the important differences between wild and managed colonies of honey bees and we will suggest ways health professionals can make use of the marvelous tools for health and survival that evolution has bestowed upon *Apis mellifera* through adaptation and natural selection.

Declines of the world's pollinators are happening at an alarming rate, and it is predicted that these declines will have adverse impacts on pollinator-sensitive commodities worth billions of dollars (Morse and Calderone 2000). The threat to the honey bee is perhaps the best understood of the pollinator declines. Its causes are diverse: widespread use of agrochemicals, loss of plant and floral diversity, invasive species, migratory beekeeping practices, and monoculture pollen sources. Furthermore, the stresses created by these environmental stressors are intensified by the honey bee's pests, parasites, and pathogens. Although no single disease agent has been identified as *the* cause of honey bee colony collapse, pests and pathogens are recognized as the primary drivers of the massive deaths of managed bee colonies worldwide. Many of these agents of disease are vectored by an ectoparasitic mite introduced from Asia, *Varroa destructor* (Ellis et al. 2010; Ratnieks and Carreck 2010).

Investigations of honey bee declines have focused primarily on the pathogens themselves and their interactions, which are now understood to be multifactorial (vanEngelsdorp et al. 2009; Becher et al. 2013; Di Prisco et al. 2016). Besides the pathogens, the environments in which honey bees live also profoundly impact colony survival. In this chapter, we will examine honey bee health and the alarming levels of colony mortality from an ecological and evolutionary perspective. We will embrace the logic of natural selection and we will learn important lessons from long-term studies of honey bee colonies living in nature (Brosi et al. 2017; Seeley 2017b, 2019a; Neumann and Blacquière 2016).

Good Genes Versus Good Lifestyle: The Varroa Story

We will begin our account of the health and fitness of wild colonies by relating the story of the Varroa mite (*V. destructor*), a parasite that switched hosts from the Eastern honey bee (*Apis cerana*) to the Western honey bee (*A. mellifera*). In order to understand the resistance to Varroa mites that is found in wild honey bee colonies, we must examine more deeply their genes and their lifestyle.

Beekeepers today rely primarily on commercial queen producers for their bee stock. Most hobby beekeepers, for example, will start an apiary or add colonies to an apiary by purchasing either a "package" of bees shipped in a cage or a nucleus colony ("nuc") living in a small hive. In North America, packaged bees are shipped from various southern states in the U.S., as well as from California, and Hawaii, so they consist of stock that is not necessarily adapted to the beekeeper's local climate, temperatures, and agents of disease. Furthermore, even though queen bees are also produced and sold across North America – their genetics often traces to just a handful of colony lines. In many places, good colony health can be fostered by the use of locally-adapted bees.

From an evolutionary perspective, the observation that wild colonies have rapidly adapted to the Varroa mite, and to the diseases they vector, over a remarkably short timeframe (ca. 10 years), suggests that surviving wild colonies have either good genes (DNA), a good lifestyle, or both (Seeley 2017a).

Good Genes

The Varroa mite is the leading cause of honey bee health problems on all beekeeping-friendly continents except Australia. Beekeepers have always experienced colony losses, but it was not until the arrival of this parasitic mite that colony die-offs became severe in North America. The Varroa mite lies at the heart of poor colony health, because it acts both as a primary stressor (the adult mites feed on the "fat bodies" of adult bees and the immature mites feed on immature bees [pupae]) and as a vector for a myriad of the viral diseases of honey bees (vanEngelsdorp et al. 2009; Martin et al. 2012). If a managed colony of honey bees is left untreated, Varroa mites will kill it within two to three years (Rosenkranz et al. 2010). Remarkably, the wild colonies living in the forests of North America today, plus some notable examples of European honey bees living on islands, are resistant to the mite (De Jong and Soares 1997; Rinderer et al. 2001; Fries et al. 2006; Le Conte et al. 2007; Oddie et al. 2017). How did this resistance evolve? We know that wild colonies in the northeastern forests of North America went through a precipitous population decline in the 1990s, following the arrival of the mite (Seeley et al. 2015; Mikheyev et al. 2015; Locke 2016). Yet, studies show that these wild colonies recovered in the absence of mite treatments without appreciable loss of genetic diversity by evolving a stable host-parasite relationship with *V. destructor*.

The genetic bottleneck associated with a precipitous population decline would have devastated most species; cheetahs and Florida panthers, to name two prominent

mammalian examples, exhibit extensive disease syndromes from low genetic variability. *A. mellifera*, however, came through its population decline with remarkable genetic variation intact because polyandry, a breeding strategy whereby the queen mates with 10–20 drones, helps maintain the genetic composition of a population. Polyandry also confers improved fitness through enhanced disease resistance (Seeley and Tarpy 2007); higher foraging rates, food storage, and population growth (Mattila and Seeley 2007); and possibly better queen physiology and lifespan in the colony (Richard et al. 2007). Fitness follows diversity and in honey bee colonies this comes through the multiple matings of the queen. In nature, there must be a trade-off between the optimal number of drone matings and the time that queens spend on their mating flights, which sometimes extend several miles from a queen bee's home. Delaplane and colleagues (2015) showed that queens artificially inseminated with sperm from 30 to 60 drones, rather than the 12 to 15 drones that are typical for the queens of wild colonies, produced more brood and had lower mite infestation rates relative to control colonies, supporting the idea that resistance to pathogens and parasites is a strong selection pressure favoring polyandry. One hypothesis to explain the high levels of polyandry of queen honey bees is that by mating with many males, the queen captures rare alleles that regulate resistance to pests and pathogens (Sherman et al. 1998; Delaplane et al. 2015). This has been confirmed in several studies in which colonies whose queens had either a high or a low number of mates were inoculated with the spores of chalkbrood (*Ascosphaera apis*) or American foulbrood (*Paenabacillus larvae*), and the levels of infection in their colonies were compared (Tarpy and Seeley 2006; Seeley and Tarpy 2007). The higher the number of mates, the lower the level of disease.

We know that Varroa mites initially killed off many wild colonies living in the forests of New York State, so maternal lines (mitochondrial DNA lineages) were lost (Mikheyev et al. 2015). Fortunately, the multiple mating by queen honey bees enabled the maintenance of the diversity of the bees' nuclear DNA despite the massive colony losses. Today, the density of wild colonies living in forests in the northeastern United States (c. 2.5 colonies per square mile, or 1 per square kilometer) is the same as it was prior to the invasion of the Varroa mites (Seeley et al. 2015; Radcliffe and Seeley 2018), and the survivor colonies possess resistance to these mites. In a comparison of the life history traits of wild colonies living in the forests around Ithaca, NY, between the 1970s (pre Varroa) and the 2010s (post Varroa), Seeley (2017b) found no differences, which implies that the wild colonies possess defenses against the mites that are not highly costly and so do not hinder colony reproduction.

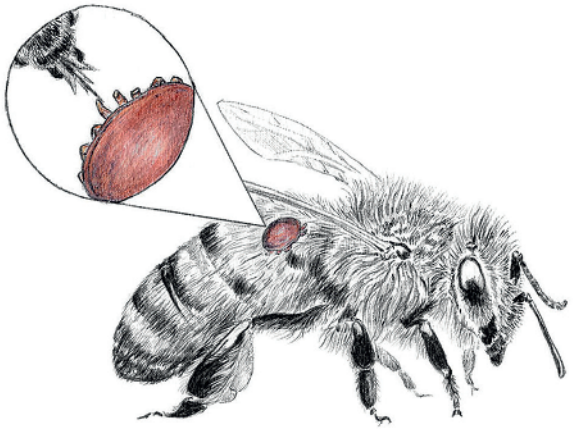

Figure 1.2 Grooming, or mite-chewing, is a heritable trait in which honey bees remove and kill adult Varroa mites by chewing off parts of the mite's body, carapace, or legs.

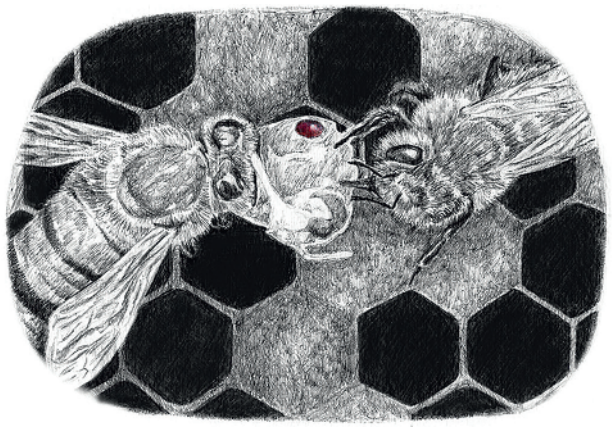

Figure 1.3 Hygienic behavior or Varroa Sensitive Hygiene (VSH), is a form of social immunity in which honey bees selectively remove the varroa-infested larvae and pupae from beneath capped cells. The mites infecting these brood cells are killed along with the developing bee upon opening of the cell.

There exist multiple mechanisms of natural Varroa resistance, a form of behavioral social immunity, that have a genetic basis. These include grooming behavior, also known as "mite chewing," and hygienic behavior, also known as Varroa Sensitive Hygiene (VSH). Grooming behavior is the process whereby worker bees kill mites by deftly chewing off the carapace, ventral plate, or legs of a mite (Figure 1.2). The strength of a colony's ability to groom Varroa mites is indicated by the percentage of chewed mites among the mites that fall onto a sticky board placed beneath a screened bottom board in a hive (Rosenkranz et al. 1997). Hygienic behavior is the process whereby worker bees remove diseased (or dead) brood from the cells in which they are (or were) developing (Figure 1.3). VSH is measured by determining the percentage of sealed brood cells that contain Varroa mites shortly

after cell capping and then again shortly before brood emergence (cell uncapping). Because this assay of a colony's VSH behavior is rather tricky to perform, people often use a different assessment of hygienic behavior: the freeze-killed brood (FKB) assay. Because the FKB assay does not involve Varroa infested brood, it is not a direct measure of VSH. The FKB assay works by freezing a c. 3 in. diameter circle of sealed brood cells, thereby killing the brood within, followed by calculating the percentage of the dead brood that have been removed, either 24 or 48 hours after the freezing of the brood (Spivak and Downey 1998).

In a long-term study in Norway, variation among colonies in their resistance to Varroa was found to be based on neither grooming behavior nor hygienic behavior, but on something else that was hindering mite reproduction. Oddie and colleagues (2017) examined managed honey bee colonies that had survived in the absence of Varroa control for >17 years alongside managed colonies that had received miticide treatments twice each year. Records were kept of daily mite drop counts, and of assays of the colonies' mite grooming and hygienic behaviors, for both survivor and control colonies. No difference was found in the proportion of damaged mites (~40% chewed in colonies of both groups) or in FKB removal rates (only ~5% brood removed). However, the average daily mite-drop counts (indicators of the mite populations in colonies) were 30% lower in surviving colonies compared to susceptible ones. Evidently, there were other colony factors (besides mite grooming and hygienic behaviors) responsible for reducing the reproductive success of the mites in these colonies of Norwegian honey bees. Since donor brood was used for the testing in both groups of colonies (mite susceptible and mite resistant), the possibility of protective traits of immature bees was eliminated. What Oddie et al. found is that in the mite-resistant colonies (but not in the mite-susceptible ones) the worker bees are uncapping brood cells and then recapping them several hours later, and that this reduces the mites' reproductive success to a level that protects the colony. An 80% reduction in mite reproductive success, together with a reduction in brood size, independent of grooming or hygienic behavior, was also described for populations of survivor (untreated) colonies of honey bees living on the island of Gotland in Sweden (Fries and Bommarco 2007; Locke and Fries 2011).

Good Lifestyle

To understand the survival of honey bee colonies living in the wild, we must look not only at their genetic makeup but also at their lifestyle. How do the ways in which wild colonies live combine with their genes to limit mite reproductive success and the virulence of mite-vectored pathogens? We know that modern beekeeping practices create living conditions for managed colonies that are far more stressful than the living conditions of colonies living in the wild (see Table 1.1). For example, we know that the artificial crowding of colonies in an apiary, the provision of large hives which foster Varroa reproduction, and the suppression of swarming behavior – are all apicultural manipulations that make large honey harvests possible for the beekeeper but are harmful to colony health (Seeley and Smith 2015; Loftus et al. 2016). Another important, but little understood, stressor experienced by managed colonies is the greater thermoregulation stresses experienced by colonies living in a standard hive compared to in a bee tree (Mitchell 2016). Our modern beekeeping practices – launched in 1852 with the invention of the movable frame hive, by Lorenzo L. Langstroth – have created new challenges for honey bee colonies, which are adapted for living without human management (interference). For the remainder of this chapter, we will explore the lifestyle features that help wild colonies of honey bees thrive despite their pests, parasites, and pathogens. We will also draw lessons that beekeepers and bee doctors can employ to help promote the health of the managed colonies living in apiaries.

Part 1: The Environment of a Wild Colony

Cavity Size

A good place to begin our exploration of wild honey bee health is understanding the home of a honey bee colony found in nature (Figure 1.4). Wild honey bees predominately make their homes inside the cavities of hollow trees, though any cavity of appropriate volume and specific characteristics will do, and this includes manmade structures, rock crevices, and other spaces. Wild colonies choose small cavities, with an average volume of just 45 l (range 30–60 l: Seeley and Morse 1976; Seeley 1977). When honey bee colonies choose their nesting sites, they seek cavities of this size, which is substantially smaller than the typical Langstroth hive in an apiary, with a volume of 120–160 l (Root and Root 1908; Loftus et al. 2016).

Nest cavity size has a major impact on honey bee health through its effect on mite population dynamics. A brief review of the Varroa life cycle will help us understand the role of nest cavity size on a colony's mite population. Varroa mites have two different life phases: the phoretic phase in which adult mites feed on the "fat bodies" of honey bees and the reproductive phase in which mites reproduce in the cells of sealed brood of workers and drones (Rosenkranz

Table 1.1 Characteristics of wild honey bees (*Apis mellifera*) that differ from managed honey bees and their impact on bee health.

Characteristic	Wild colonies	Reference	Managed colonies	Reference
Colony lifespan	Long-lived 5–6 yr once established	Seeley (2017b)	Short-lived; 2–3 yr without miticides	Rosenkranz et al. (2010)
Annual survival	High survivorship 84% (established) 20% (founder)	Seeley (2017b)	Low survivorship (0–50%)	Ellis et al. (2010)
Cavity size of home	Small cavity; 45 l (30–60 l)	Seeley and Morse (1976)	Large cavity; 120–160 l	Loftus et al. (2016)
Swarming frequency	87% annual queen turnover in established colonies	Seeley (2017b)	Swarming suppressed, so low queen turnover	Oliver (2015)
Propolis barrier	Complete barrier "propolis envelope"	Seeley and Morse (1976)	Incomplete barrier smooth hive walls	Hodges et al. (2018)
Colony spacing	Colonies far apart (~1 km)	Seeley and Smith (2015) Radcliffe and Seeley (2018)	Colonies close together (~1 km)	Root and Root (1908)
Virulence level	vertical transmission of mite-vectored pathogens, via swarming	Seeley and Smith (2015)	Virulence favored by horizontal transmission of mite-vectored pathogens, via drifting/robbing	Seeley and Smith (2015)
Nest insulation	Thick-walled (20 cm/8-in.) well insulated tree cavity	Seeley and Morse (1976)	Thin-walled (2.5 cm/1-in.) poorly insulated Langstroth	Root and Root (1908)
Immune Function	Strong social immunity, Immune genes downregulated	Simone et al. (2009)	Weak social immunity, Immune genes upregulated	Borba et al. (2015)

et al. 2010). Only adult female mites are phoretic; both the tiny males and the nymphal stage females remain within the capped brood cells. Honey bee larvae are essential for the mite because it has no free-living stage off the host – the mite is entirely dependent on honey bee brood for its own propagation. Honey bee colonies living in large hives hold more brood than those living in natural nest cavities, so colonies in large hives are especially favorable for mite reproduction.

All honey bee populations that have survived for more than a decade without miticide treatments share a common feature: their colonies are small (Locke 2016). Small colony size relates directly to the dynamics of brood development and swarming. Having relatively few brood has two significant impacts on mite reproduction. First, since Varroa mites only reproduce within the cells of sealed (pupal stage) brood, the reproduction of these mites is hampered by the relatively small brood nests of wild colonies. Second, a small nest cavity size shortens the time before the sealed brood fills a colony's brood nest, and this brood nest congestion is one of the primary cues for swarms and afterswarms (Winston 1980). When colonies living in large hives (two deep hive bodies plus two honey supers) were compared to colonies living in small hives (just one deep hive body, to mimic the nest cavity size in nature), it was found that the small-hive colonies had reduced mite loads and improved colony survival, as a result of more frequent swarming and lowered Varroa infestations (Loftus et al. 2016).

Wall Thickness and Thermoregulation

Seeley and Morse (1976) reported that the average wall thickness of natural nest cavities is approximately 20 cm (~8 in.). The wall thickness of a standard Langstroth hive is just 1.9 cm (0.75 in.), hence some 10 times thinner than the nest cavity wall of a bee tree. The reduced wall thickness in Langstroth hives creates a large reduction in nest insulation, possibly resulting in adverse effects on colony energetics. Large temperature fluctuations inside a hive exacerbate colony stress by increasing the demands on colony nutrition and hydration (more nectar and water foraging trips), by impairing a colony's ability to maintain thermal homeostasis (more fanning and "bearding" when it is hot, and more metabolic heat production when it is cold), and by hastening entry into a winter cluster – all of which increase the physiological demands on the colony (Mitchell 2016).

Coombs et al. (2010) found that natural tree cavities buffered environmental temperatures such that tree cavities were cooler than ambient during the day and warmer than ambient during the night. During the day, the tree diameter at breast height was the most important variable determining

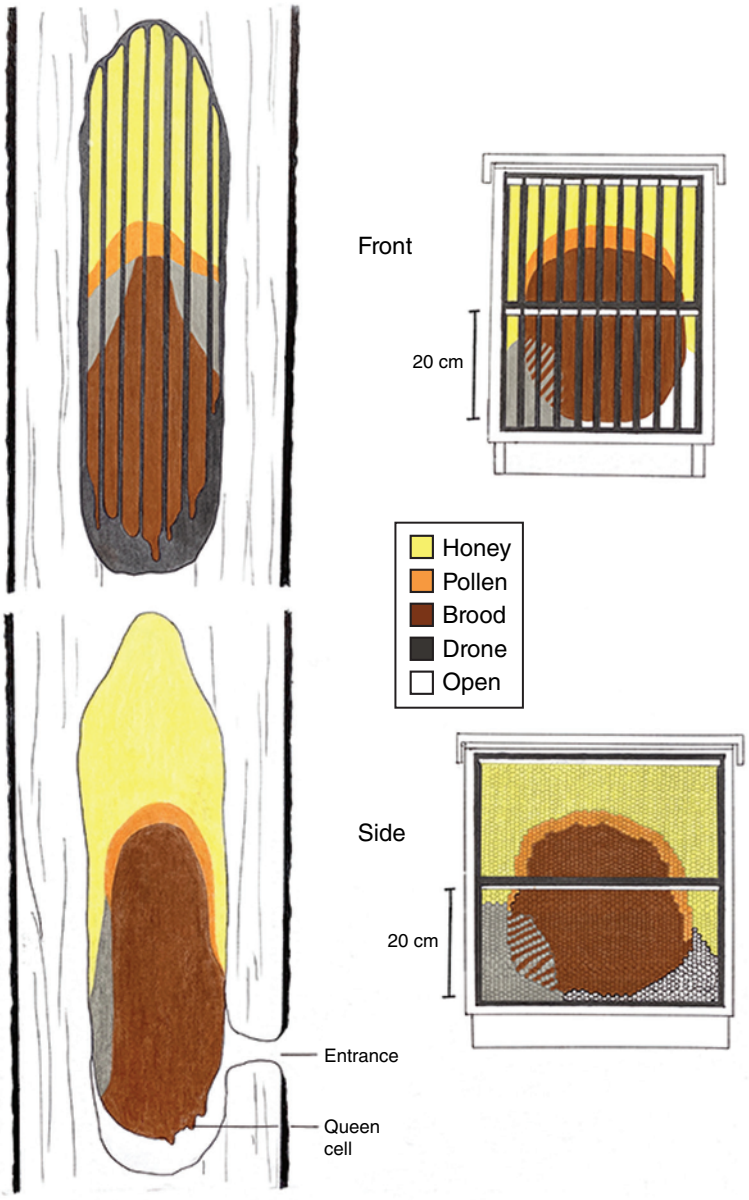

Figure 1.4 An illustration comparing the structure and organization of a honey bee nest as found in a bee tree (left) and a standard Langstroth hive made up of two deep hive bodies (right). The colors correspond to brood and hive products. A typical bee tree cavity has a volume averaging 40 l, whereas two deep hive bodies have a volume of 80 l. These differences in cavity volume are directly correlated with the size of a colony's brood nest and varroa reproductive success.

cavity temperature. At night, diameter and tree health were important with large *living* trees offering the most stable thermal environment. We compared the ambient temperatures inside two tall, man-made cavities; one was inside a rectangular wooden box (built of 1.9 cm thick pine boards, as used for Langstroth hives) and the other inside a living sugar maple tree (*Acer saccharum*) (Figure 1.5). These two cavities were built with the same dimensions (24 cm × 24 cm × 87 cm), which mimicked those of a typical tree cavity of a wild colony [see Tree Beekeeping by Powell (2015)]. Temperature recordings over a year revealed striking differences in interior temperature dynamics between the two cavities. In the poorly insulated box, the temperature closely followed the ambient temperature; the thin walls provided little or no temperature buffering. In the tree, though, the temperatures varied much less; they did not reach the extreme highs and lows found inside the uninsulated box (Seeley and Radcliffe unpublished data; see Figure 1.6a,b).

Mitchell (2016) found that heat is transferred four to seven times faster across the thin walls of a traditional hive relative to the walls of a natural (bee tree) enclosure. To maintain a colony's cluster core temperature of 35 °C (the set point of the brood nest), any energy lost through transfer from the hive walls must be replaced through the bees'

Figure 1.5 A research station beside the Shindagin Hollow State Forest in upstate New York. It was designed to test the environmental fluctuations – temperature (°C) and relative humidity (%) – inside two cavities of identical dimensions but with walls of different thicknesses, c. 2 cm vs. 20–30 cm. One (a) is a wooden box with walls like those of a Langstroth hive and the other (b) is a live sugar maple tree (*Acer saccharum*) in which a typical size bee cavity was cut using a chainsaw and adze. *Source:* Photo by Robin Radcliffe.

metabolic activity (bees isometrically contract their flight muscles to generate heat). Mitchell predicted that colonies living in hives (or trees) providing well-insulated cavities will not need to assemble into tight clusters until the ambient temperature is below 0°C. Mitchell concluded that the high thermal insulation of nests in bee trees results in increased relative humidity inside the cavity, decreased reproduction by Varroa mites, and enhanced survival of honey bee colonies.

Propolis Envelope

Propolis ("bee glue") is a resinous substance collected by honey bees from the buds and wounds of trees. When combined with beeswax, it makes a cement that bees use to fill the crevices and coat the walls of their nest cavities, often completely enshrouding their nests. This coating of the walls, floor, and ceiling of the nests of wild colonies with tree resins makes a "propolis envelope" that can be 2–3 mm thick (Seeley and Morse 1976). The propolis lining of the nest cavity probably serves several functions: creating a solid surface for comb attachment, reducing cavity draftiness, enhancing nest defense, waterproofing, and bolstering a colony's defense against microbial infections.

Ancient Greeks used propolis to treat abscesses, Assyrians put it on their wounds, and Egyptians used it for embalming their dead. Although humans have long recognized the health benefits of propolis for its antiseptic, anti-inflammatory, antibiotic, antifungal, anesthetic, and healing properties, only in the last century have humans discovered the specific compounds that give propolis its medicinal value – of the more than 180 compounds identified in propolis to date, one group (a class of plant-based polyphenols known as flavonoids) are of particular interest for their protective antioxidant properties. These same compounds that mankind values in propolis also confer health benefits to the honey bee colony through social immunity – a collective behavioral defense that produces

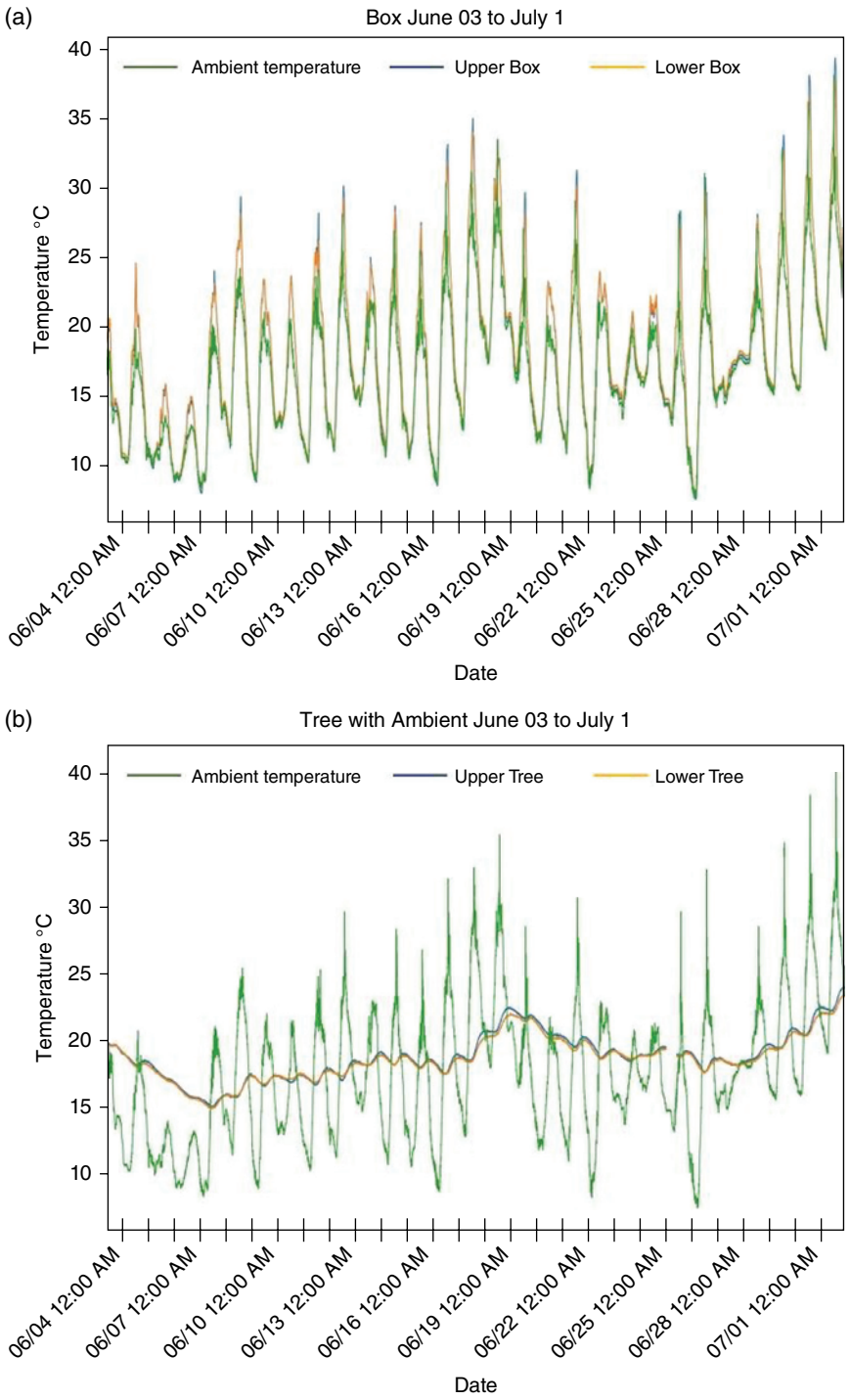

Figure 1.6 A month-long comparison of temperatures (°C) inside a thin-walled nest cavity made of 1.9-cm-thick lumber (a) and inside a thick-walled cavity made in a living sugar maple tree (*Acer saccharum*) having a wall thickness of 20–30 cm (b). Each cavity had two temperature probes, located c. 10 cm from either the floor or the ceiling of the cavity. In both figures the green line represents the ambient environmental temperature, while the orange and blue lines are the probes located within the respective cavities.

colony-wide immunity that in turn reduces the expression of immune genes in individual bees (Borba et al. 2015).

Curiously, the use of propolis for colony defense is limited to the temperate regions of the world. Neither the tropical honey bees in Asia (*A. cerana, Apis florea*, and *Apis dorsata*) nor those in Africa (the African subspecies of *A. mellifera*) make use of propolis other than for structural purposes (Simone et al. 2009; Kuropatnicki et al. 2013). It is the European honey bees living in nature for which the collection and use of propolis for its colony-level immunoprotective effects has reached its highest expression. Yet, rather than being viewed as a specific compound to be cultivated, propolis is more often than not regarded as an annoyance by modern beekeepers. Beekeepers are constantly scraping off propolis as they remove frames to manipulate their colonies. And the Langstroth hive bodies used by the vast majority of beekeepers today lack the rough inner surfaces of a bee tree or other natural cavity that stimulate propolis deposition by foragers. Colonies managed by beekeepers are not strongly stimulated to collect and use propolis. Indeed, it is the complex surface of the natural cavity that provides the tactile stimuli necessary for the deposition of propolis as a hive barrier by worker bees, something almost entirely lacking in modern hives made from smooth planed lumber (Hodges et al. 2018). Hodges and colleagues investigated three methods to increase the textural complexity of the interior surface of a standard hive body; these methods included using plastic propolis traps stapled to the inside wall surfaces, cutting horizontal parallel saw kerfs that were 7 cm apart and 0.3 cm deep, and roughening of the interior wall surface using a mechanical wire brush. The three interior hive wall types were compared to an unmodified, smooth-walled hive by measuring the bees' propolis application. Although the colonies were not challenged with specific pathogens, all three texturing methods induced significantly more propolis deposition compared to controls. The authors concluded that using unplaned, rough lumber for the interior hive surfaces would increase propolis deposition over standard hives built using lumber that is planed smooth on both sides.

A curious observation arising out of the mapping of the honey bee genome was the discovery that honey bees possess just one-third of the genes coding for immune function typically found in solitary insects (Evans et al. 2006; Honey Bee Gene Sequencing Consortium 2006). It was hypothesized that the weak capacity for an immune response in individual honey bees might be compensated by behavioral or colony-level defenses, or a form of social immunity. Indeed, as social insects, honey bees are steadfastly hygienic by removing alien organisms that gain entry to the nest, by feeding young bees antimicrobial products, by creating compounds that offer barriers to infection, and evolving complex interaction networks that serve to compartmentalize infections. The first indication that the bees' nest environment could influence immune expression in honey bees was discovered by Simone et al. (2009). Honey bees living in hives whose inner walls were coated with propolis extracts (derived from resins found in Minnesota and Brazil) invested less energy on immune function compared to bees living in hives without such coating. The colonies living in the propolis enriched hives also had lower bacterial loads. Scientists believe that individual bees are not immunocompromised, but rather that they conserve energy by not upregulating their immune genes except when a pathogen is encountered. This means that the defenses provided by social immunity (e.g. the collection of tree resins for propolis) allows individual bees to divert energy resources from immune function to other hive activities such as nursing, wax building, and foraging. This strategy likely maximizes the health and fitness of the entire colony.

Bee Microbiome

An oft-overlooked aspect of the bee environment that is essential to the good lifestyle of honey bees is their microbiome, that is, the community of specialized microbes (bacteria and yeasts) that have coevolved to live inside the bees and in their nests (e.g. in their pollen stores). We again return to the tenet of our chapter: the need to learn about the honey bee's natural biome to understand its biology, including its relationships with its pathogens. The honey bee microbiome is remarkable in that it is nearly consistent across thousands of individuals from hive to hive and even across continents. The honey bee's microbiome is similar to that of humans in that both feature specialized bacteria that have coevolved with their host and are socially transmitted (Engel et al. 2012; Zheng et al. 2018). Honey bees are first inoculated with bacteria in the larval stage, presumably through the food provided by nurse bees. However, during pupation, when bees undergo the final phase of metamorphosis, a bee's exoskeleton (including the gut lining and any associated bacteria) is shed in a process known as ecdysis. Therefore, honey bees emerge as young adults without a gut flora, except for those microorganisms they pick up when chewing through the wax cappings of their cells. The characteristic microflora of a worker bee is, therefore, developed mainly following emergence and through direct social interactions with conspecific worker bees. By four to six days of age, the population of a worker bee's gut flora stabilizes at 10^8–10^9 bacterial cells.

Although both wild honey bees and those living in apiaries possess complex microbiomes, some beekeeping

practices – such as feeding pollen substitutes and treating with antibiotics – can alter the microflora of honey bees (Fleming et al. 2015; Maes et al. 2016). Dysbiosis, or unhealthy shifts in gut microflora, was observed in bees consuming aged pollen or pollen substitutes and was linked to impaired larval development, increased bee mortality and infection with pathogens such as *Nosema* and *Frischella*. Raymann et al. (2017) observed considerable changes in the gut microbial community composition and size following treatment with tetracycline, the most commonly used antibiotic in beekeeping operations globally. The authors concluded that decreased survival in honey bees was directly attributed to increased susceptibility to infection by opportunistic pathogens that colonized the gut after antibiotic use. The honey bee microbiome is thought to promote bee health and development in several ways. Gut microbes are required for normal bee weight gain, an effect which can be attributed to regulation of endocrine signaling of important bee hormones. The microbiome increases the levels of vitellogenin and juvenile hormone in worker bees, and these regulate the nutritional status and the development of their social behaviors, so it is likely that the state of the bees' microbiomes affects the health of the whole colony. Bee microbes are also implicated in modulating the worker bee's immune system (Zheng et al. 2018).

Alterations in the microbiota of the bee gut have been linked to disease and reduced fitness of the bee host. The use of tetracycline – an antibiotic commonly used to treat American foulbrood and European foulbrood, and often given prophylactically – reduces both the number and the composition of normal bacteria in the bee gut. Raymann and colleagues (2018) found that *Serratia marcescens*, a known pathogen of honey bees and other insects, normally inhabits the bee gut without eliciting a host immune response. However, bee disease occurs when this pathogen is inoculated into a bee's hemolymph through the bite of a Varroa mite or when the gut microbiome is disturbed with antibiotic use. Researchers studying Colony Collapse Disorder observed a shift in gut pathogen abundance and diversity, and proposed that such shifts within diseased honey bees may be a biomarker for collapsing colonies (Cornman et al. 2012). See Chapter 9 for more details on the bee microbiome.

Part 2: Epidemiology for Bee Health: How Lifestyle Impacts Disease Spread

The preceding comparison of the environments of honey bee colonies living in the wild versus in apiaries sets the stage for reviewing the host–parasite interactions that ultimately define colony health. Let us now compare the impacts of disease on colonies living in the differing settings in which honey bee colonies now find themselves. Compared to organisms that do not live in large and complex eusocial societies (i.e. ones with a reproductive division of labor and overlapping generations) honey bees have far greater complexities in their host–pathogen and host–parasite relationships.

Ecological Drivers of Disease

Living in crowded communities of thousands of individuals, honey bees interact closely through regular communication behaviors, grooming activities, and the trophallactic transfer of food and glandular secretions. This complex group living provides abundant opportunities for pathogens to spread and reproduce. Moreover, the high temperature and high humidity of a honey bee colony's home makes it a perfect environment for disease outbreaks. It comes as no surprise, then, that many of the protective mechanisms that honey bees have evolved to control the spread of disease operate at the level of the whole colony, the superorganism. The members of a colony work together closely to achieve a *social immunity*: they groom themselves and one another (allogroom); they work as undertakers to remove dead and diseased bees; they collect antibiotic enriched pollen and nectar; and they practice miticidal and hygienic behaviors by biting off the body parts of mites and by removing infected bee larvae and pupae from their nests (Fries and Camazine 2001). Relatively few mechanisms of disease resistance have evolved at the level of the individual bee. These include individual immune system functioning and filters in the proventriculus (the valve between esophagus and stomach) that remove spores of American foulbrood. Most of these protective mechanisms limit *intra*-colony transmission of disease agents, and they work well. What is probably the primary driver of disease problems for honey bees at present, however, is *inter*-colony disease transmission.

A Critical Distinction: Vertical vs. Horizontal Disease Transmission

The *method* by which a disease is transmitted from colony to colony is a fundamental determinant of pathogen virulence. Vertical transmission (the spread of disease from parent to offspring) favors the evolution of avirulence whereas horizontal transmission (the spread of disease among unrelated individuals) favors the evolution of virulence (Lipstich et al. 1996). This is because pathogens and parasites that spread vertically need their host to stay healthy to produce offspring, whereas those that spread horizontally do not have this need. Although numerous

other host factors (i.e. host longevity, density, population structure, and novel hosts) and pathogen factors (i.e. vector availability and pathogen replication potential) also influence virulence, we will focus on how the mode of honey bee pathogen and parasite transmission within and among colonies impacts the evolution of the virulence of these agents of disease.

Vertical Transmission: Swarming

In honey bees, one way that a colony achieves reproductive success is by swarming: an established colony casts a swarm to produce a new colony. The other way that a colony achieves reproductive success is by producing drones; even though weak colonies can propagate their genes by producing drones, this does not create another colony. If a pathogen or parasite that is transmitted vertically (from parent to offspring) weakens its host and so hampers it from producing offspring (which for honey bee colonies equates to casting swarms) then it reduces its own reproductive success. In short, the natural mode of colony reproduction in honey bees favors the evolution of avirulence in most of its pathogens and parasites. The two exceptions to this generalization are American foulbrood and *Varroa destructor*, both of which are easily transmitted horizontally when one colony robs honey from another.

Swarming also helps inhibit the reproduction of Varroa mites (and other agents of brood diseases) by creating a natural break in brood production, which forces the mites to likewise suspend their reproduction (Seeley 2017b). Once a daughter queen emerges to replace the mother queen that has left in a swarm, this daughter queen must leave the hive to fly to a drone congregation area, where she will mate with multiple drones before returning to the hive to commence egg laying. This transition from mother queen to daughter queen creates a period without sealed brood (needed for mite reproduction) that can last from 7 to 14 days. This imposes a break in the reproduction of the Varroa mites. Furthermore, with each swarming event a sizable fraction (approximately a third) of the colony's mite population is exported with the departing workforce: the fraction of mites shed can be readily calculated since about half of the female breeding-age mites are on the workers in a colony at any given time, and nearly three-quarters of these workers depart in the prime swarm (Rangel and Seeley 2012). In a six-year study of the life-histories of wild honey bee colonies living in a forest in the northeast US, Seeley (2017b) found that most (~87%) swarmed each summer.

In contrast to the relatively small nest cavities of wild honey bee colonies, the colonies kept by beekeepers occupy large hives, and they are less apt to produce swarms (Oliver 2015). The swarm control methods of beekeepers include transferring sealed brood to the top of the hive and queen exclusion (the Demaree method), cutting out queen cells, preventing the filling of cells around the brood nest with nectar (possibly a cue for swarming) by providing empty combs above the brood nest, reversing the brood boxes and inserting empty combs in the brood nest, and reducing the worker populations of colonies by splitting them. All of these methods weaken the stimuli that trigger swarming, but only one helps control the Varroa mites: the removal of bees. We propose instead controlled colony fission by making "splits" to mimic the beneficial effects of swarming on mite control (Loftus et al. 2016).

Horizontal Transmission: Bee Drift, Robbing, Forager Contact, and Contamination

Fries and Camazine (2001) outline three distinct things that a pathogen must do to reproduce and disperse to a new honey bee colony. A pathogen must: (i) infect a single honey bee; (ii) infect multiple honey bees; and (iii) infect another colony. Of these, it is the spread to *another colony* that should most concern beekeepers and bee doctors:

> In terms of fitness, the successful transfer of a pathogen's offspring to a new colony is a critical step in its life history. If a parasite or pathogen fails to achieve a foothold in another host colony, the parasite will not increase its reproductive fitness, regardless of how prolific it has been within the original host colony. Thus, hurdles #1 and #2 (intra-individual and intra-colony transmission) are important aspects of pathogen fitness only to the extent that they contribute to more efficient inter-colony transmission (Fries and Camazine 2001).

The transfer of pathogens or parasites from one colony to another horizontally can occur by four main routes: drifting, robbing, contact while foraging, and shared use of a contaminated environment. Drifting occurs when a forager enters another colony by accident, something that is largely a byproduct of modern apiary management since the wide spacing of wild colonies largely precludes drifting (Seeley 2017b; Seeley and Smith 2015). Robbing occurs primarily during periods of a nectar dearth, when strong colonies attempt rob honey from weak ones. In this case, pathogen transfer is most likely to occur from the weak colony to the strong colony, though the opposite is also possible. The transfer of pathogens during contact while foraging has been described in both natural and experimental models, including video documentation of a Varroa mite jumping onto a foraging honey bee the

instant the bee lands on a flower (Peck et al. 2016). Finally, diseases can be spread from one colony to another through sharing of contaminated water, as has been observed with infections of the microsporidium *Nosema apis* (L'Arrivée 1965).

Honey Bee Demographic Turnover

In the article entitled, *What epidemiology can teach us about honey bee health management*, Delaplane (2017) reviewed the ecological and evolutionary impacts of the host–parasite relationship and proposed that an important driver of virulence is the high rate of introduction of susceptible colonies into apiaries (i.e. the introduction of new individuals into existing populations). Epidemiologists recognize three distinct "compartments" for individuals in a population exposed to a disease: Susceptible (S), Infected (I), and Recovered (R) individuals. In the simplest SIR (Susceptible, Infected, and Recovered) model, once susceptible animals catch the disease they become members of the infected "compartment" and can spread the disease to susceptible individuals. The infected animals that survive then move into the recovered "compartment" and are considered immune for life (Kermack and McKendrick 1927). Delaplane argues that the beekeeping practice of restocking "dead-out" hives with nucleus colonies prolongs the epidemic by introducing new "S" individuals into the population of colonies in an apiary, a process that fosters the evolution of virulence (Fries and Camazine 2001). In a closed population, however, a disease epidemic is not artificially prolonged and the surviving individuals tend to have resistance, so there tends to be coevolution of the host–parasite or host–pathogen relationship. Given the high levels of colony losses experienced by beekeepers each year, the restocking of colonies with "nuc" replacements – thereby introducing a fresh batch of susceptible individuals to the apiary population – may represent one of the most noteworthy (and easy to address) management practices contributing to the collapse of honey bee colonies (Cornman et al. 2012).

Now let us return to those curious observations of populations of mite-surviving honey bee colonies in various places around the world. A common thread among these reports of populations of honey bee colonies surviving Varroa infestation for long periods without the use of miticides is the *isolation* of these populations of colonies from managed colonies. The colonies live on islands (Gotland Island in Sweden or the island of Fernando de Noronha off the coast of Brazil), in remote inaccessible regions (far-eastern Russia), or in an intact forest ecosystem (the Arnot Forest in the northeastern United States). The isolation from managed colonies found in all three of these scenarios must have favored the evolution of avirulence of Varroa and the multitude of viral diseases vectored by this mite. In essence, these populations all lack an important feature that drives virulence of infectious disease – a steady introduction of "S" individuals. With no new "Susceptible" colonies coming into these populations, in each case the mites and the bees have co-evolved a stable host–parasite relationship. In the case of the Arnot Forest bees, we know the Varroa invasion was associated with significant loss of genetic diversity in the bees (an indicator of heavy colony mortality caused by Varroa), but at the same time the surviving colonies of this population possessed effective defenses against the mites (Mikheyev et al. 2015; Seeley 2017b).

It is here that the "good lifestyle" of colonies occupying small nest cavities, living widely spaced, and swarming frequently meets the "good genes" of colonies that are living as an isolated "island" of colonies. Now that we have married the good genes and the good lifestyle aspects of health in our examination of honey bee management, where does the bee doctor fit into this picture? In the final section of our chapter, we will explore how we can use the knowledge garnered from a deep understanding of wild colonies to develop a new way of keeping healthy colonies in managed apiaries, an approach recently named Darwinian beekeeping (Seeley 2017a).

Lessons from the Wild Bees

Modern apiarists practice pest/disease control, close colony spacing, swarm control, queen rearing, mating control (sometimes), annual requeening of colonies, migratory beekeeping, queen imports, drone reduction, and various other alterations of the bee's natural biology. These apiculture practices tend to limit natural selection and to disrupt the hard-won adaptations of *A. mellifera*; they impact both the genes and the lifestyle of the honey bee (Neumann and Blacquière 2016). Now, what can be done from an animal husbandry and animal health perspective to reverse such trends?

The bee doctor must be prepared to examine honey bee health through a new lens that takes a holistic approach to medicine – one that features an understanding of and appreciation for the health of honey bees living in nature. In some parts of the world, beekeepers are already looking at beekeeping less as a process of domestication that forces the production of honey, wax, propolis, and pollination at great cost to colonies and more as the stewardship of a natural living system. The global decline in bee health is a direct consequence of man's disruption of this system: the

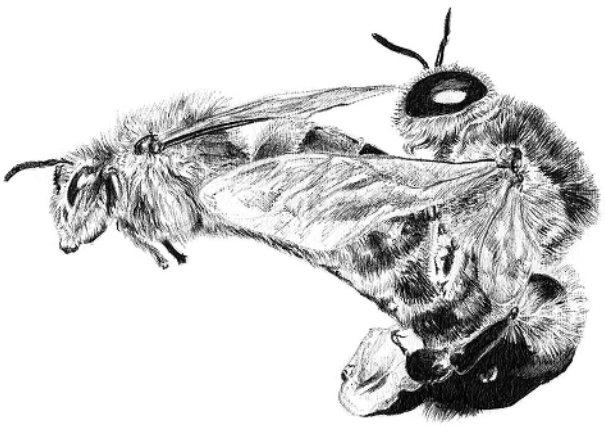

Figure 1.7 Polyandry, or the multiple matings of a queen with drones from different patrilines, has been associated with colony vigor and improved winter survival. The health benefits of polyandry are linked to improved foraging rates, greater brood production, lower mite infestations, and the possession of rare alleles important for control of infectious disease.

introduction of exotic parasites and pathogens, the rise in disease virulence driven by beekeeping practices, and the evolution of drug resistance caused by indiscriminate treatments of colonies. Indeed, it is the pharmaceutical-centric approach to preventative care for honey bees that is the fundamental reason behind the inclusion of honey bees among the food-producing animals in North America that now fall under FDA regulations requiring the services of a veterinarian for antibiotic use. A key feature of a healthy system is achieving a balance between the host and the pathogen that promotes host resistance and pathogen avirulence – we can find this balance by promoting good genes and a good lifestyle in the bees.

Promoting Good Genes

The idea that honey bees have been domesticated by mankind remains a matter of debate. What is clear is that across North America there are populations of wild colonies of *A. mellifera* that thrive independent of beekeeping activities and that do not require the regular input of new colonies from honey bee swarms arising from managed colonies (Oliver 2014; Seeley 2017b; Radcliffe and Seeley 2018). Furthermore, the wild colonies tend to be genetically distinct from those that queen breeders produce for commercial purposes; the former are both more diverse genetically and they show strong evidence of regional adaptation (Figure 1.7) (reviewed in Seeley 2019a,b). Evidently, the honey bee colonies managed by beekeepers are semi-domesticated, since their genes are influenced somewhat by queen breeders and their lifestyle is strongly influenced by their owners (Chapman et al. 2008; Oliver 2014).

An important lesson can be learned from the many animals that man has domesticated over the past thousand years: domestication carries with it a reliance on humans and generally a loss of the ability to survive in the wild. Here we can take some guidance from Charles Darwin:

> One of the most remarkable features in our domesticated races is that we see in them adaptation, not indeed to the animal's or plant's own good, but to man's use or fancy. Some variations have probably arisen suddenly, or by one step. However, we cannot suppose that all the breeds were suddenly produced as perfect and useful as we now see them. . . . The key is man's power of accumulative selection: Nature gives successive variations: man adds then up in certain directions useful to him. (Darwin 1868)

Among the honey bee traits that are known to have a genetic basis, resistance to disease has shown to be a strong component of colony fitness (Tarpy and Seeley 2006). With this in mind, we believe that both the beekeeper and the bee doctor will be wise to consider the following items when it comes to managing the genetics of honey bees.

Goal 1: Select Locally Adapted, Survivor Stock

Bait hives are a ready method for beekeepers to incorporate wild honey bees and their genes into their apiaries (Figure 1.8). A queen honey bee of local origin is well suited to an ecoregion or ecotype and has genes that provide a good fit with the local floral diversity, regional environmental conditions (including extremes of temperature, humidity, drought, etc.), and agents of disease.

A wonderful example of the adaptation of honey bees to their locale is the ecotype of *A. mellifera* that lives in the Landes heathlands of southwestern France (Louveaux 1973). The Landes bees have evolved to have a brood cycle with an unusual, second peak of brood production in August, just in time for the bloom of ling heather (*Calluna vulgaris*) in the Landes landscape. It is interesting to note that when Louveaux moved Paris honey bees to Landes, the Paris bees kept ahead (in colony weight gain, pollen collection, and brood production) of the Landes bees until the middle of July. Up to that point, the Landes bees had trailed behind the Paris bees because the Paris bees had reared more brood in May and June. In August, however, all the colonies of the Landes bees had a second burst of young bees emerging shortly before the heather bloom and by the end of summer these colonies had collected an astonishing 14 kg more honey than the colonies of Paris bees (Louveaux 1973).

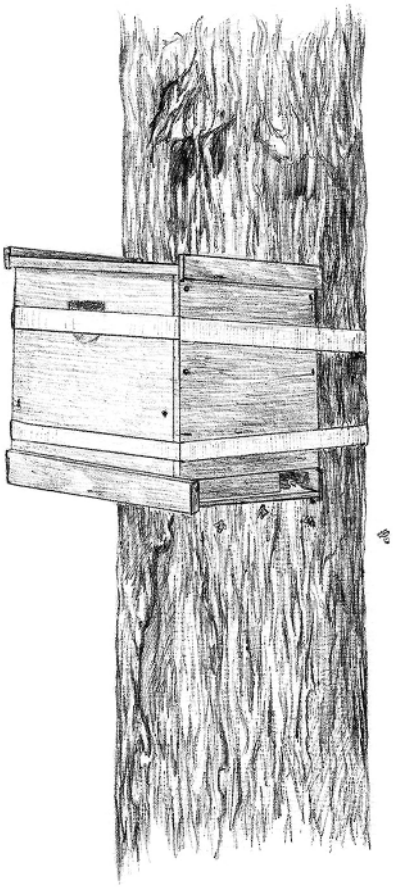

Figure 1.8 Bait hives are small nest boxes that are filled with empty comb and sometimes lures or attractants (lemon grass oil or Nasanov pheromone). During the swarm season (May to June in the northeastern United States), scout bees search out and select bait hives during house hunting behaviors. Wild honey bees are well adapted to Varroa and often fare much better than managed bees. Therefore, bait hives are a simple means for the acquisition of locally adapted honey bee stock when they are used in places where there are few beekeepers.

Goal 2: Promote Drone Comb Building and Drone Mating in Congregation Areas

Modern apiarists work to limit the amount of drone comb produced by honey bees because beekeepers have learned that by preventing their colonies from producing drones, they can increase honey production (Seeley 2002). Furthermore, drone comb is the preferred site for *Varroa* reproduction. Limiting it, however, partially "castrates" a colony by reducing the ability of a colony to contribute to the population of drones in a region, an important driver of honey bee diversity and fitness (Seeley 2017b). It is now known that a colony's health and productivity is enhanced by its having high genetic diversity among its worker bees, which arises from the multiple mating (polyandry) strategy of queen honey bees (Tarpy and Seeley 2006; Seeley and Tarpy 2007; Mattila and Seeley 2007). On average, a queen honey bee mates with, and acquires sperm from 10 to 20 drones. Inhibiting drone production in colonies hinders the maintenance of genetic diversity within a region, including the genes that may hold resistance to mites (Rosenkranz et al. 2010).

Goal 3: Cull Failing Colonies Before Collapse

Some veterinarians with experience in honey bee disease and/or epidemiology have campaigned against the emergence of Treatment-Free Beekeeping or Natural Beekeeping because of the risk of spreading disease through the collapse of colonies. Perhaps most alarming is the phenomenon of "mite bomb" colonies (ones collapsing from high mite loads) that spread mites and virulent strains of the Deformed Wing Virus to neighboring colonies (Martin et al. 2012). When Varroa mites reached Hawaii, Martin and colleagues observed a drastic increase in the prevalence of DWV from 10% to 100% (the percentage of honey bee colonies infected with DWV virus), a millionfold increase in DWV viral copies in infected bees, and a reduction in DWV diversity to a single highly contagious strain. A collapse of 274 of 419 managed colonies on Oahu Island followed. The beekeeper should either treat Varroa-infested colonies once a critical mite infestation level is reached (typically c. three mites per hundred bees sampled) or cull (euthanize) highly infested colonies before they can spread their mites to neighboring colonies or surrounding apiaries.

Goal 4: Select Quality Queens and Let the Bees Requeen!

A vigorously laying queen is the most efficient promoter of good genes, so it is of utmost importance to keep colonies headed by highly fertile queens. If a hive must be requeened, it is better to allow the bees to choose their new queen (if age-appropriate larvae are present) than to replace her artificially since it has been shown that when bees are confronted with an emergency need for queen rearing, they do not select larvae at random for their queen cells (as a beekeeper might), but instead select larvae of certain patrilines (Moritz et al. 2005). In the future, beekeepers and bee doctors may be able to better assess queen quality through quantitative means; queen quality, judged in terms of body weight, is a good predictor of a queen's mating flight number, ovarian size, and overall mating success (Amiri et al. 2017).

Although insects lack the immunological memory provided by the antibodies of vertebrates, queen bees can recognize specific pathogens and prime their offspring against them (Salmela et al. 2015). The queen passes these immune signals to her future offspring via the egg-yolk vitellogenin, a protein that has been shown to bind harmful bacteria, including the *P. larvae* of American foulbrood. Queens of

local origin will pass onto their larva the essential immune cells that are adapted to the pathogens she has encountered in her environment, giving her offspring the chance to build defenses against disease agents before they (the bees) emerge and become exposed to pathogens in the nest.

Promoting Good Lifestyle

The ways in which honey bee colonies live in the wild differ substantially from those experienced by colonies living in apiaries, where they are managed by beekeepers for honey production or crop pollination. Although there is debate about whether honey bees are truly domesticated (modified genetically to be more useful to humans), it is certain that humans have changed their living conditions through a variety of means. Just as domestic animals are manipulated by farmers in their housing, feed, and even medical care, so too are the colonies of honey bees that are managed by beekeepers. We suggest the following goals to help improve colony fitness through alterations of honey bee lifestyle.

Goal 1: Boost Rather than Disrupt Social Immunity of the Superorganism

In the next chapter we will learn that a honey bee colony is a superorganism. In other words, it is a highly integrated unit of function that has been shaped by natural selection to function as an integrated whole. One result of this high level of organization is that the immune system of a worker honey bee is relatively simple compared to those of non-social bees. With this in mind, we should note that there the beekeeper and bee doctor can inadvertantly weaken the social immunity of the colony. Perhaps the most damaging is breaking and reducing the propolis envelope, which will impair the colony's social immunity and compromise honey bee health. Therefore, the number of times a hive is opened for inspections or manipulations should be reduced to a minimum. The layers of propolis lining the walls and inner cover are playing an important role and should be left intact. The beekeeper can stimulate his/her bees living in a hive to build a complete propolis envelope by using hives whose inner walls have been roughened or by lining the interior surfaces with propolis collection screens.

Goal 2: Quarantine from Pests and Pathogens

Bee doctors should work closely with beekeepers to avoid bringing honey bee colonies from an outside location into an established apiary. The most important drivers of honey bee die-offs in North America have all been caused by emerging pests and pathogens that came from other parts of the world – Varroa mites from Asia, small hive beetles from Africa, and both chalkbrood fungus and acarine mites from Europe (Seeley 2017b). Returning to the SIR model, it follows that beekeepers should reduce as much as possible the introduction of new colonies that represent the "Susceptibles" into an apiary. If these introduced colonies are exposed to or are carrying a novel pathogen, then they can produce outbreaks. Specifically, Delaplane (2017) warns against bringing in outside bees to replace dead outs and recommends instead that these apiary losses should be replaced by splits made within the same apiary. Loftus et al. (2016) found in their study of the effects of colony size and frequent swarming on resistance to Varroa that 60 m was not a sufficient distance between apiaries to avoid spread of Varroa between apiaries during a nectar dearth. Three of the 12 small-hive colonies in this experiment suddenly acquired high mite loads when one of the large-hive colonies collapsed in the adjacent apiary. Evidently, robbers from these three small colonies brought home Varroa from the large colony that was collapsing, resulting in their own collapses several weeks later. It is therefore recommended that introducing new colonies to an apiary be done only after an appropriate period of quarantine in a separate location at least 1 km away.

Goal 3: Design Apiary as Close to Nature as Feasible

The idea that the "design" of an organism is a product of natural selection, which favors survival and reproduction, is the foundation for modern biology and is the basis for Darwinian beekeeping. The fitness of a honey bee colony is directly related to its ability to survive as a healthy unit and to cast viable swarms and produce fertile drones. It follows that we should aim to help our colonies survive and reproduce, if we want them to be part of a healthy population in the area. This viewpoint is perhaps the most challenging for the beekeeper to adopt because it is, in a sense, a break from managing colonies to maximize their production of goods (honey) and services (pollination). If, however, our goal as beekeepers and bee doctors is to sustain populations of healthy colonies of bees, then we should consider making changes in bee management practices that are in keeping with wild colony biology (Seeley 2017b):

First, keep the number of hives in an apiary to a small number to reduce crowding. High colony density promotes robbing and drifting, and thus the mixing of pathogens among host colonies. This mixing ("horizontal transmission") can favor the evolution of virulence in pathogens and eventually lead to the collapse of colonies.

Second, keep hive size small to avoid creating colonies with large brood chambers that support large, continually running "assembly lines" of mite reproduction. Seeley (2017b) suggests using one deep hive body for a brood nest

and one shallow super over a queen excluder for harvesting some honey.

Third, perform colony splits (as a method to mimic swarming behavior) to initiate a broodless period that creates a break in reproduction by Varroa mites (Loftus et al. 2016). A beekeeper makes a split (a small, new colony) by removing from a colony its queen and some of its worker bees and brood, and putting them in a separate hive. The remainder of the colony, still living in the original hive, then rears a replacement queen.

Fourth, space colonies as widely as possible (>10 m) and face their hives in different directions to reduce the drifting of returning foragers into the hives of neighboring colonies (Seeley and Smith 2015). Artistic beekeepers can also color code their hives or add unique graphic designs (geometric shapes of color work well!) above the hive entrance to help the bees orient back to their own hives. The anatomy and physiology of the bee, which will be outlined in future chapters, will help guide the beekeeper in choosing colors and patterns most suitable to optimize color and shape recognition by returning bees. Honey bees discriminate colors across the range of green to ultraviolet. Hives painted red appear black to bees, and is a poor choice for hive color given that it is the color of a key predator – the black bear – therefore, hives painted in shades of yellows, greens, blues, or pastel colors are more easily distinguished by honey bees compared to ones painted red or purple.

Fifth, hives should provide the bees with a well-insulated nesting cavity, so that less of a colony's energy is expended on heating and cooling, to achieve thermal homeostasis. The health of a honey bee colony depends on keeping its brood nest at ca. 35 °C from spring to fall, and to keeping the outer layer of the winter cluster above about 10 °C throughout winter.

Finally, bee doctors should avoid treatment of pathogens without a clear diagnosis. A key component of the honey bee environment is the bee's microbiome, which is hidden from view to anyone without a microscope and culture plate. The social behaviors that produce the characteristic flora of the honey bee's gut serve important roles in prevention of disease; the indiscriminate use of antibiotic therapy is known to promote resistance as well as alter the symbiotic gut microbes that underlie the health of honey bee colonies.

Charles Darwin marveled at the honey bee organism and spent a great deal of time studying the organization and structure of their colonies, including the wonderous design of their hexagonal comb. Darwin could not have known the full extent of the threats that the world's honey bees would face in the twenty-first century – from climate change to mite-vectored pathogens. But perhaps he had the bees in mind when he wrote: *It is not the strongest of species that survives, nor the most intelligent, but the one most responsive to change*.

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