



Overwintering honey bees: biology and management

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In temperate climates, honey bees (*Apis mellifera*) survive the winter by entering a distinct physiological and behavioral state. In recent years, beekeepers are reporting unsustainably high colony losses during the winter, which have been linked to parasitization by *Varroa* mites, virus infections, geographic location, and variation across honey bee genotypes. Here, we review literature on environmental, physiological, and social factors regulating entrance, maintenance, and exit from the overwintering state in honey bees in temperate regions and develop a testable model to explain how multiple factors may be acting synergistically to regulate this complex transition. We also review existing knowledge of the factors affecting overwintering survival in honey bees and providing suggestions to beekeepers aiming to improve their colonies' overwintering success.

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Introduction

Honey bees (*Apis mellifera*) live in a wide geographic range occupying various climatic regions and facing different challenges in different parts of the world and times of the year [1]. Winter is the greatest challenge to the honey bee colonies in temperate regions. The honey bee is one of the few insect species that is adapted to survive winter conditions without becoming completely dormant, entering a distinct physiological and behavioral state [2]. Before *Varroa* and tracheal mites were introduced, overwintering colony losses were ~10% [77]. However, an average loss of ~30% winter loss was reported by the US beekeepers in surveys since 2006, compromising sustainability of commercial beekeeping operations [3*,49]. Honey bees provide critical pollination services for natural and agro-ecosystems world-wide. Successful overwintering of honey bee colonies is critical to meet the pollination

needs of early spring-blooming crops such as almonds, apples and cherries. Indeed, the February/March California bloom requires 1.7 of the 2.5 million US honey bee colonies to pollinate 860 thousand acres of almonds, and this demand is projected to continue to rise. This demand could be met, at least in part, by improved overwintering: a 10% reduction in overwintering loss could provide an additional 250 000 colonies for early spring pollination.

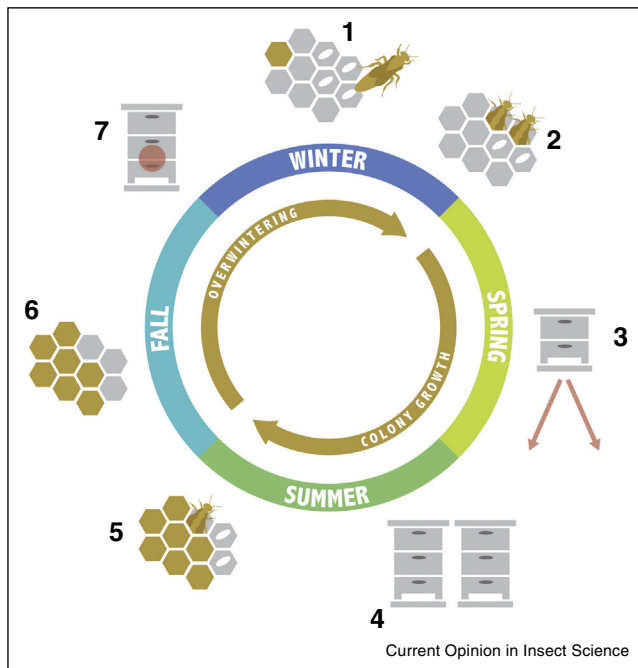
The overwintering state of a honey bee colony is characterized by changes in the behavior and physiology of individual bees, including reduced individual activity, changes in endocrine profiles, increased nutrient stores and increased longevity, as well as changes at the colony level, including cessation of brood rearing and most remarkably, formation of a thermoregulating cluster. In non-temperate parts of the world, there are seasonal factors other than winter (i.e. dry, rainy, or hot periods with little forage available) seem to trigger a broodless state followed by absconding of the nest site in search of a new one [78]. While certain aspects of the two life cycle events are similar (i.e. lack of forage, decreased/halted foraging, and cessation of brood rearing), overwintering provides a unique challenge for the honey bees to overcome; extreme cold.

Previous studies have demonstrated that levels of *Varroa* mites, viruses, geographic location, and genotype are correlated with winter colony losses [4–9]. However, the effects of these factors on overwintering bees at the molecular, physiological and behavioral level have not been comprehensively studied, making it challenging to develop better management approaches to improve overwintering success. Here, we describe our existing knowledge of the environmental and physiological factors regulating entrance, maintenance, and exit from the overwintering state in honey bees in temperate regions. We then discuss factors associated with overwintering losses. Finally, we synthesize this information to provide recommendations for beekeepers to maximize overwintering survival and highlight areas of future investigation.

Overview of the annual colony cycle in temperate regions

Honey bee colonies exhibit distinct seasonal states (see Figure 1) [2]. In temperate climates, brood rearing starts in winter (when the average maximum ambient temperature is as low as 4 °C), peaks in spring, decreases through summer, and ceases in early fall [6,10,11**,76]. Brood build-up in the spring typically leads to swarming, where the majority of the workers leave the colony with the old queen in search of a new nest site, leaving behind a new

Figure 1



Honey bee colony life cycle. Hexagonal patterns represent cells in combs. Gray cells are empty, brown cells represent food stored (honey and/or pollen), and white elliptic figures in the cells represent eggs. Brood rearing starts in winter (1) and peaks in spring (2). The rapid increase in worker population in spring results in swarming (3). After swarming, both colonies rebuild their worker populations and forage to increase their food stores through summer (4). Brood rearing decreases by the end of summer (5) and ceases in fall (6), with the production of the winter bee cohort. In the winter, worker bees form a thermoregulating cluster (red circle inside the hive) with the decrease in ambient temperature (7).

*Graphical design by Harland Patch and Nick Sloff, Penn State. HPG — After Snodgrass, 1925. Vitellogenin — Heli Hvukainen, used with permission.

queen and the remaining workers to rebuild the original colony [12,13]. After swarming, both original and new colonies spend the remainder of the summer and early fall collecting pollen, which is used as a protein source for brood rearing, and nectar, which is converted to honey and used as a general energy source especially during winter months [14]. When the temperature drops below 10 °C, the bees in the colony form a thermoregulating cluster [15]. Clustering bees vibrate their flight muscles to generate heat that maintains an outer edge temperature higher than 6 °C, usually ~12 °C. This ensures that the bees on the outermost edges of the cluster do not cool below their viable temperature. When brood rearing is initiated in winter, the cluster surrounds the brood area and maintains the core temperature at ~33 °C [16]. This thermoregulation is achieved only when the cluster is in a confined space, as in the case of natural or manmade hives.

Overwintering behavior and physiology of worker honey bees

The behavior of individual bees in the colony also changes dramatically as the colony moves through the different seasons [17]. In late spring, summer, and early fall, workers are short-lived (~30 days) and exhibit an age-based division of labor. The youngest bees, generally <10 day old, perform nursing tasks, middle age bees between 10 and 20 days old engage in tasks such as comb building, food storage, guarding and undertaking, while the oldest bees in the colony serve as foragers [18]. In the fall, as brood rearing declines, the long-lived (up to 8 months) bees that will survive the winter are produced (hereafter referred to as ‘winter bees’) [19**]. These winter bees form the thermoregulating cluster when temperatures drop. Once brood rearing re-initiates in late winter/early spring, the division of labor resumes among overwintered worker bees [10].

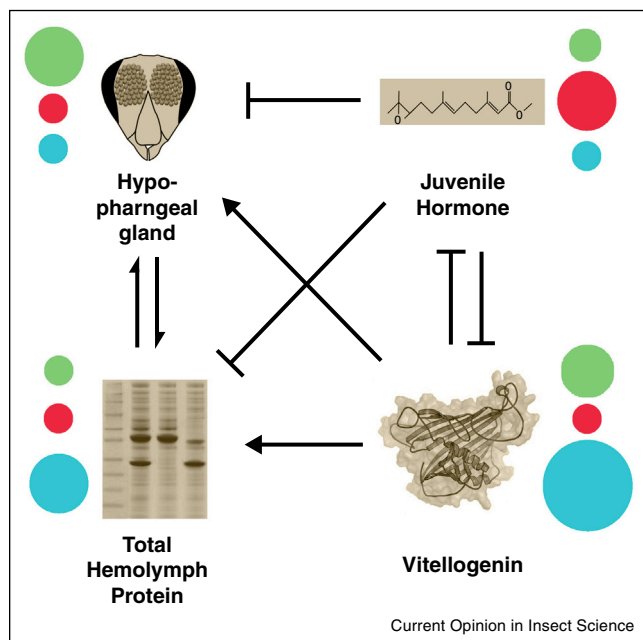
In addition to these profound differences in behavior, honey bees also exhibit dramatic physiological changes across the seasons. Levels of juvenile hormone (JH), vitellogenin (Vg), and hemolymph proteins as well as the size of hypopharyngeal gland (HPG) are correlated with and regulate each other, and vary significantly between nurse, forager, and winter bees (see Figure 2). Briefly, JH levels are low in nurse and winter bees, and higher in foragers. In contrast, levels of Vg and hemolymph proteins are significantly higher and HPG is larger in nurses and winter bees than foragers.

Though the physiological differences in nurse bees, forager bees and winter bees are well documented, few studies have examined changes in physiology of bees over the course of the winter. Fluri *et al.* [19**] marked newly emerged bees in fall (beginning of September) and then sampled them in January and February to compare levels of JH, Vg, total protein, and HPG weight. Interestingly, there were no significant differences between the bees in fall and mid-winter or late-winter. These results suggest that bees produced in the fall are already in the ‘winter’ physiological state, and this state remains stable throughout the winter. Using a broader range of timepoints, Huang and Robinson [20] found that the JH biosynthesis rate decreases from early October to mid-November, reaches its lowest level in mid-January, and then steeply increases in February and March. The early spring rise in JH titers correlate with a decrease in Vg levels, hemolymph protein levels, and a HPG size; thus, bees that overwintered return to the forager bee physiological state in spring [19**].

Factors that trigger transition to and exit from the overwintering state

Several environmental cues associated with seasonal changes, such as photoperiod, temperature, and nutritional state, have been examined to determine if these trigger

Figure 2



Interaction of key physiological factors in nurse, forager and winter bees. Colored disks on the side of each factor represents the relative abundance of the factor in nurse bees (green), forager bees (red), and winter bees (blue). As bees transition from nursing to foraging, juvenile hormone (JH) levels in the hemolymph rise. In workers, Vg serves as a nutrient storage protein and is involved in a negative feedback loop with JH — as JH levels raise, Vg levels decrease, and decreasing Vg results in increasing JH. Total hemolymph protein levels — which include Vg and all other types of proteins — are higher in nurse bees than in forager bees. Finally, hypopharyngeal glands are located in heads of worker bees and produce the secretions (brood food) fed to other members of the colony; HPG size is largest in nurse bees.
*Graphical design by Harland Patch, Penn State.

seasonal changes in worker behavior and physiology and overall colony behavior. Cherednikov [21] found that artificially shortening the day length in late spring and summer causes several changes in treated colonies versus control colonies, including: cannibalization of brood and eggs, increased collection and consumption of nectar and pollen, greater accumulation of fatty tissues in workers, increased resistance of workers to cold and starvation, and aggregation at ambient temperatures as high as 18–20 °C. Fluri and Bogdanov [22] confirmed that shortened photoperiod causes an increase in both lipid and protein content of the worker bees' fat body (the tissue which primarily regulates metabolism and nutrient storage), resulting in a more 'winter-like' physiology in worker honey bees. However, artificial shortening of photoperiod did not result in the appearance of long-lived winter bees in Fluri and Bogdanov's study, and Cherednikov did not measure this variable. Since decreasing the daylight hours would also result in restricting foraging behavior (which is likely an important factor in triggering the production of

winter bees, see below), it would be valuable to test the effects of artificially lengthening daylight period to determine if this delays the timing of production of winter bees in the fall.

Temperature has also been investigated as a factor that can trigger overwintering changes in bees. Moving a summer colony to a cold room resulted in a significant decrease in both biosynthesis rates and titers of JH in foragers within 8 days [20]. However, it was not explored if this was a direct result of temperature and/or photoperiod cues (these colonies were also kept in the dark) or an indirect effect due to associated decreases in nutrition, brood rearing, or restricted foraging activity. It is unlikely that a rise in the ambient temperature serves as the environmental cue to commence the brood rearing after the winter break, since brood rearing stops in October and November but restarts in the much colder December and January [23]. However, it is possible that different factors serve to trigger the production of overwintering bees and the exit from overwintering in the spring. Again, experiments that uncouple light and temperature cues and monitor impacts on the timing of winter bee production can greatly improve our understanding of the regulation of this process.

Changes in availability of nutritional resources can shift the timing of the entry to and exit from the overwintering state. Increasing pollen stores through supplementary feeding or restricting pollen stores by placing pollen traps across the colony entrances will, respectively, delay or accelerate the timing of the production of long-lived winter bees relative to control colonies [24**]. Supplementing colonies with pollen in the spring also stimulates brood production [25**]. However, though nectar and pollen can vary widely in nutritional quality and diverse floral resources are optimal for honey bees and other pollinators (Vaudo *et al.*, in this issue), the effects of nutritional quality on overwintering behavior and success remain to be determined.

These effects of nutrition may be due to indirect effects on brood production. Pollen serves as the primary source of protein and lipids, which are critical for brood rearing [14]. Honey bees adjust the rate of brood rearing according to the availability of protein resources and cease reproduction when they are completely deprived [24**,26,27]. When broodless winter colonies were transferred into a flight room and fed pollen, the queens immediately initiated egg-laying and workers activated their HPGs in 3–4 days [28]. Moreover, workers in free-flying colonies in the summer will physiologically resemble winter bees when the colonies are made broodless [19**,28–30]. Thus, lack of brood alone can induce colonies to transition to their wintering physiological state, regardless of photoperiod, temperature, or availability of floral resources.

It is unclear how brood triggers the winter-like physiological changes in worker bees. Bühler *et al.* [31] demonstrated that when a micro-climate resembling the brood nest (35 °C, 1.5% CO₂) is artificially created in broodless colonies, JH titers of workers rapidly rise. Additionally, the presence of brood can change the circadian rhythm of worker bees; broodless colonies exhibit a prominent circadian rhythm in their activity, while colonies with brood are less rhythmic [32,33]. Developing larvae also produce brood pheromone, which elicits a multitude of behavioral and physiological responses in worker bees and could be a key factor in overwintering behavior (reviewed in Grozinger, in press). Brood pheromone triggers pollen foraging by forager bees, which, as noted above, will in turn stimulate brood rearing and the production of brood pheromone [34,35].

Brood pheromone and its components can accelerate maturation and the transition to foraging in middle aged bees [36,37]. If bees are reared in the absence of brood or brood pheromone, they have higher levels of Vg when they are middle-aged (7–20 days old), initiate foraging later, and live longer, even under winter-like conditions [38**]. Thus, it seems likely that brood pheromone exposure *prior to* the production of overwintering bees or *after* brood rearing is initiated in the spring would be beneficial (because it stimulates brood production and colony growth), while brood pheromone exposure during the middle of the winter may trigger early maturation of the winter bees and be detrimental.

Foragers also release a pheromone (ethyl oleate) which, like brood pheromone, impacts behavioral maturation of young bees. However, ethyl oleate will slow down the transition to foraging, and maintain bees in the nursing state longer [39,40]. Restricting the flight of foragers so they remain in the colony will slow behavioral maturation of young bees, presumably by increasing their exposure to ethyl oleate [41,42].

Integrating the available information, we suggest that the entry and exit to the overwintering state is mediated by interactions between environmental nutritional resources, brood/brood pheromone levels, forager pheromone, and potentially temperature and photoperiod cues. During the fall, there is a decrease in available foraging resources which, in combination with days getting shorter and colder, results in a decrease in foraging effort in colonies. This should result in more foragers staying in the colony, and an increase in the exposure of young workers to ethyl oleate, which should slow their behavioral maturation. Additionally, the reduction in pollen foraging should decrease levels of brood production and brood pheromone, which also slows the behavioral maturation of workers. With reduced brood pheromone, foraging is further reduced, further amplifying the effects of ethyl oleate. In the late winter/early spring, day length

increases sufficiently to trigger the production of a small amount of brood. The presence of brood pheromone stimulates behavioral maturation in some of the worker bees, results in the generation of a foraging force reversing the process that has happened in fall. Brood pheromone also stimulates the collection of pollen by these foragers, once temperatures are high enough. The influx of pollen into the colony further stimulates brood production and facilitates rapid colony growth in spring.

Factors impacting overwintering losses

Multiple factors can impact colony survival during the winter [73]. Based on surveys of US beekeepers, reasons for winter colony losses vary by operation size: backyard beekeepers generally blame weak colonies in fall, starvation, queen failure and *Varroa* mites, while commercial beekeepers blame queen failure, *Varroa* mites, pesticides and colony collapse disorder [43]. Furthermore, colony loss during winter is not uniform through US, with some geographic regions experiencing greater losses than others. However, the different average winter losses among states cannot be fully explained by latitudinal differences [43]. Below, we review recent research studies that sought to examine several of these factors and their correlations with colony loss, and recommend management practices to mitigate the effects of these factors.

Large-scale assessments of beekeeper-managed colonies in Canada [44] and Germany [6] found that levels of *Varroa* mites most strongly correlated with overwintering losses (also see [74]). *Varroa* mites are ectoparasites of honey bees which feed on the hemolymph of developing pupae [45]. *Varroa* feeding negatively impacts many of the physiological changes associated with overwintering, including decreasing Vg titers [46], reducing abdominal protein and carbohydrate levels [47], and reducing lifespan in both summer and winter bees [48]. Smaller experimental studies found a significant reduction in overwintering losses when colonies are treated with miticides to reduce *Varroa* levels [7–9]. Self-reporting by beekeepers indicated that beekeepers that employed *Varroa* mite control products had ~40% loss while those who did not experienced ~60% loss [49]. However, miticide use to control *Varroa* can also negatively affect bees [50], and thus we recommend using an ‘integrated pest management’ approach to regulate *Varroa* mite populations and improve overwintering success (see recommendation 3).

In studies in which viral infections were monitored, infection with deformed wing virus (DWV), Israeli Acute Paralysis virus (IAPV), and acute bee paralysis virus (ABPV) were also strongly correlated with colony survival [6,7,9,51,75]. *Varroa* mites transmit viruses and reduce their host’s immunocompetence, resulting in increased viral loads [52–54]. Thus, it remains to be determined to what extent the correlations between *Varroa* loads and

colonies losses are due to the effects of *Varroa* or viruses. Viruses alone, however, have been shown to have dramatic impacts on honey bee physiology and behavior, including accelerated maturation, though effects on nutritional stores, Vg levels, and other aspects of overwintering physiology have not been fully examined ([75], reviewed in [McMenamin and Genersch, in this issue](#); [Flennikan et al., in this issue](#)). To reduce viral titers and protect immunocompetence of honey bees, beekeepers should aim to minimize *Varroa* mite populations and reduce exposure to other stressors, such as pesticides, which can increase viral titers (see recommendations 3 & 5) [55].

The genetic background (genotype) of the colony may also influence overwintering success. In a large study spanning 16 different genetic stocks and 20 apiaries across Europe, survival was significantly impacted by both location and genotype [56^{••}]. Furthermore, colonies headed by local queens survived an average of 83 days longer than colonies headed by non-local queens. Thus, the results suggest there can be local adaptation of genotypes to their environments. However, while there is evidence for distinct populations/subspecies of honey bees in Europe [57], US honey bee populations are likely more genetically heterogeneous, given extensive migratory beekeeping practices (more than 60% of US colonies are moved to California for almond pollination, for example) and the nationwide shipping of thousands of queens and package bees produced in the southern US and California. Recent studies from our group have not found any evidence for local adaptation of honey bees stocks to winter conditions (Doke, Frazier and Grozinger, in prep). However, studies from a SARE-funded farmer grant conducted in Maine, compared 50 colonies established from package bees, half requeened with local survivor stock while the other half were not requeened. Eighty percent of the requeened colonies survived the winter while only 28% of those that were not requeened survived. These results are consistent with two previous studies by the same group [58]. However, it is unclear if the effects are due to local adaptation of the survivor stock, or simply that queens produced by local beekeeping operations are of higher quality than those produced by large-scale commercial rearing operations. Indeed, there can be substantial queen effects on the entry into the overwintering state and colony winter survival as requeening in mid-summer effects when the winter bees are first observed in colonies [11^{••}]. Thus, if beekeepers need to requeen their colonies, they should consider requeening during summer using queens from high quality stocks and breeders (see recommendations 1 & 2).

Colony size and nutritional stores are also expected to impact colony survival. Larger colonies use their stored food more efficiently as *per capita* food consumption is lower in large colonies compared to small ones [59] and colonies which are larger entering the winter are more

likely to be successful exiting the winter and ultimately produce greater annual honey yields (Farrar, 1952 as reviewed in [6]). Indeed, recent studies from our group showed that fall weight was an important indicator of winter survival; 90% of colonies ≥ 22.5 kg survived, while only 27% of those ≤ 18 kg survived (weights expressed here exclude the weight of woodenware associated with hives; Doke *et al.*, unpublished data). Interestingly, supplementing or restricting colonies' access to pollen in fall do not impact the number of winter bees produced, their physiological nutritional stores, or their performance [27,60^{••}], and thus factors other than fall nutrition (perhaps genotype and/or parasite/pathogen loads and/or resource availability throughout the season) seem to regulate the population size and performance of winter bees [73]. Thus, beekeepers should facilitate brood rearing throughout the spring, summer and fall by maintaining colonies in nutrient rich locations and/or supplementing nutrition, and combine small colonies early in the fall. Additionally, when colonies are treated with synthetic brood pheromone in fall, they consume more protein supplement and brood production and colony growth increase [61]. Year-long brood pheromone treatment of colonies results in increased honey production, colony size and overwintering success [62]. Thus, if it is not feasible to support the colonies through these means, brood pheromone treatment may be an option to facilitate colony growth and increase chances of successful overwintering (see recommendation 1).

Honey bee exposure to pesticides has recently been better documented ([63–65]; Mullin, 2015, [Berenbaum, in this issue](#); [Long and Krupke, in this issue](#)). Mullin *et al.* [63] found miticides used for *Varroa* control to be the most prevalent residues in wax, enhancing the chances of resistance in *Varroa* against these chemicals while these and other identified agrochemicals increase the potential for pesticide contamination of hive products. However, fungicides were found at the highest levels in pollen and residues of all pesticide classes were found in samples of wax and pollen. The impacts of pesticide (active ingredients and formulation materials) exposure have been associated with numerous sublethal effects including reduced longevity, impaired immune function, learning and memory, orientation, foraging and motor coordination [66–70]. While these impacts likely compromise the ability of colonies to successfully overwinter, little work has been done to examine pesticide effects on overwintering success. Nonetheless beekeepers should attempt to minimize honey bee colony exposure to pesticides (see recommendation 5).

Conclusions and future directions

Overwintering in honey bees is a complex process, which integrates multiple environmental cues, social cues and interactions within the colony, and physiological and molecular changes in individual bees. Using the available

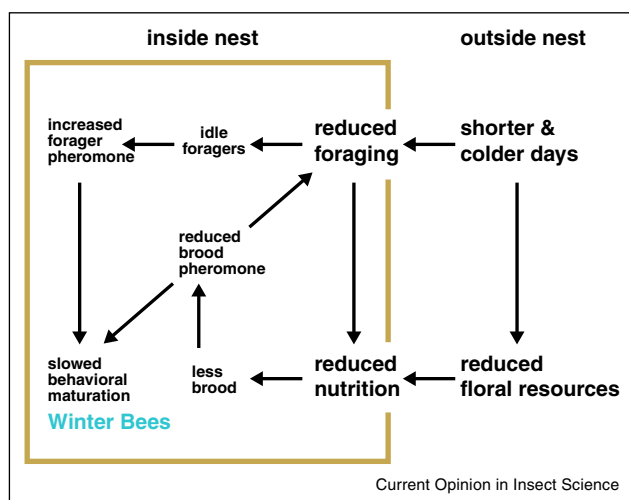
information, we have developed a model which explains how the entry, maintenance and exit from overwintering may be regulated by these factors (see Figure 3), but further studies are necessary to comprehensively test this model by uncoupling and individually testing these factors, many of which are closely correlated. Furthermore, it remains to be determined whether ‘winter bee’ development is triggered during larval stages or adult stages, or both. We have also developed recommendations that should improve overwintering survival, some of which are well tested (integrated pest management approaches to *Varroa* control), while others remain to be fully explored (effects of brood pheromone exposure and pesticides). With the development of genomic tools and approaches that will allow us to understand how and when the genome of individual bees responds to seasonal cues and conditions (Grozinger and Robinson, in this issue), our understanding of this complex process can be vastly improved, and with it our ability to better manage honey bee colonies.

From the bench to the beehive: science-based recommendations for best management practices for beekeepers

Efforts to reduce overwintering losses should focus on enhancing colony strength and food stores in fall, improving queen quality and protecting bees from *Varroa* mites, pathogens and pesticides.

1. **Only strong colonies with large populations of workers and abundant food stores should be overwintered.** Weak colonies, showing no symptoms of disease, should be

Figure 3



Proposed model for regulation of production of winter bees. The production of winter bees is likely regulated by interactions among temperature and photoperiod cues, environmental nutritional resources, brood/brood pheromone levels, and forager pheromone (ethyl oleate). See text for details.*Graphical design by Harland Patch, Penn State.

combined early in the season. To build strong colonies that are both productive and well-positioned to survive winter, select apiary locations with abundant and diverse sources of pollen and nectar throughout the season. Sufficient stores of honey and pollen are vital and quantities needed for overwintering vary depending on geographic location. If honey stores are inadequate, colonies can be fed a 2:1 (granulated sucrose: water) syrup in early fall to bring them up to a desired weight while minimizing the energy spent by the workers for removal of moisture, which would be the case if a less concentrated syrup was preferred (e.g. 1:1). High-quality pollen substitutes are also available if pollen is in short supply. However; diverse, pesticide-free, natural pollen is considered optimal.

2. **Only colonies with young, high-quality queens should be overwintered.** Poor-laying or old queens should be replaced with young queens, ideally reared from *Varroa* resistance or survivor stock, prior to winter.
3. ***Varroa* mites should be monitored and controlled using Integrated Pest Management (IPM) tactics.** Resistant or survivor stocks can be considered a first line of defense. Other techniques such as drone brood removal and screen bottom boards have delivered mixed results but are likely advantageous when integrated as part of an overall IPM approach. Monitor mite levels throughout the season using alcohol or soapy water wash, sugar rolls or sticky boards and keep mites below an established threshold. Thresholds will vary depending on geographic location, beekeeper tolerance for chemical use as well as risk of losing colonies. In general, if mite levels reach a threshold of ≥ 5 –10 mites per 300 bees in mid-late summer, a control chemical should be considered, before the winter bee population is produced. There is evidence of widespread resistance to coumaphos [71] and fluvalinate [72], for this reason beekeepers are encouraged to consider other alternatives.
4. **Wind breaks and insulation can be beneficial as long as proper ventilation is provided.** Honey bee colonies require varying degrees of protection because winter conditions vary dramatically across the US. Wind breaks are advantageous in locations with prominent cold winter winds. In geographic regions with long harsh winters, overwintering can be improved when colonies are wrapped, insulated or overwintered indoors [79]. Other considerations include providing good hive ventilation to reduce moisture build-up and condensation and entrance screens of ¼ in. hardware cloth to keep out invading mice.
5. **All beekeepers, but particularly those engaged in crop pollination, should take steps to protect bees from pesticide exposure, including those used for mite control.** If colonies are used for pollinating multiple crops, allow them to recover on high-quality forage between pollination contracts or consider a rotation scheme where half are pollinating while half are

allowed to recover, then reverse. Feeding a high-quality pollen substitute during crop pollination may dilute the effects of pesticide-tainted pollen. Good communication with growers and pesticide applicators is critical to minimize pesticide exposure. Additionally, reducing the excessive use of *Varroa* control chemical and eliminating use of off-label products will minimize their build-up in wax. Lastly, all beekeepers should have a comb-culling program in place to minimize the build-up of pesticides and pathogens.

Efforts to improve overwintering success of honey bee colonies will require the attention of beekeepers from the time colonies come out of winter in early spring until they are 'put to bed' for winter. Additionally, these efforts will depend upon on-going research to develop a deeper understanding of the complexities of honey bee overwintering behavior and physiology.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Ruttner F: *Biogeography and Taxonomy of Honeybees*. Springer-Verlag; 1988.
2. Winston ML: *The Biology of the Honey Bee*. Harvard University Press; 1991.
3. Lee KV, Steinhauer N, Rennich K, Wilson ME, Tarpay DR, Caron DM, Rose R, Delaplane KS, Baylis K, Lengerich EJ et al.: *A national survey of managed honey bee 2013–2014 annual colony losses in the USA: results from the Bee Informed Partnership*. 2015. The Bee Informed Partnership provides information on annual colony losses and surveys beekeepers for potential causes and management approaches to mitigate these causes.
4. Pirk CWW, Human H, Crewe RM, vanEngelsdorp D: *A survey of managed honey bee colony losses in the Republic of South Africa – 2009 to 2011*. *J Apic Res* 2014, **53**:35-42.
5. Meixner MD, Francis RM, Gajda A, Kryger P, Andonov S, Uzunov A, Topolska G, Costa C, Amiri E, Berg S et al.: *Occurrence of parasites and pathogens in honey bee colonies used in a European genotype–environment interactions experiment*. *J Apic Res* 2014, **53**:215-229.
6. Genersch E, Ohe W, Kaatz H, Schroeder A, Otten C, Buechler R, Berg S, Ritter W, Muehlen W, Gisder S et al.: *The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies*. *Apidologie* 2010, **41**:332-352.
7. Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P: *Predictive markers of honey bee colony collapse*. *PLOS ONE* 2012, **7**:e32151.
8. Nazzi F, Brown SP, Annoscia D, Del Piccolo F, Di Prisco G, Varricchio P, Vedova GD, Cattonaro F, Caprio E, Pennacchio F: *Synergistic parasite–pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies*. *PLoS Pathog* 2012, **8**:e1002735.
9. van Dooremalen C, Gerritsen L, Cornelissen B, van der Steen JJM, van Langevelde F, Blacquièrre T: *Winter survival of individual honey bees and honey bee colonies depends on level of Varroa destructor infestation*. *PLOS ONE* 2012, **7**:e36285.
10. Seeley T, Visscher P: *Survival of honeybees in cold climates: the critical timing of colony growth and reproduction*. *Ecol Entomol* 1985, **10**:81-88.
11. Mattila HR, Harris JL, Otis GW: *Timing of production of winter bees in honey bee (Apis mellifera) colonies*. *Insectes Soc* 2001, **48**:88-93. This study demonstrates that short-lived worker bees in colonies are gradually replaced by long-lived winter bees starting in August, and the timing of the turn-over can be influenced by factors within the colony.
12. Grozinger CM, Richards J, Mattila HR: *From molecules to societies: mechanisms regulating swarming behavior in honey bees (Apis spp.)*. *Apidologie* 2014, **45**:327-346.
13. Seeley TD: *Honey Bee Democracy*. Princeton University Press; 2010.
14. Nicolson SW: *Bee food: the chemistry and nutritional value of nectar, pollen and mixtures of the two*. *Afr Zool* 2011, **46**:197-204.
15. Phillips EF, Demuth GS: *Temperature of the honeybee cluster in winter*. *Bull US Dept Agric* 1914, **93**:1-16.
16. Moeller F: *Overwintering of honey bee colonies*. *USDA Prod Res Rep* 1977, **169**:1-15.
17. Page RE, Christine Y, Peng S: *Aging and development in social insects with emphasis on the honey bee, Apis mellifera L.* *Exp Gerontol* 2001, **36**:695-711.
18. Robinson GE: *Regulation of division of labor in insect societies*. *Annu Rev Entomol* 1992, **37**:637-665.
19. Fluri P, Lüscher M, Wille H, Gerig L: *Changes in weight of the pharyngeal gland and haemolymph titres of juvenile hormone, protein and vitellogenin in worker honey bees*. *J Insect Physiol* 1982, **28**:61-68. Authors demonstrated that many of the physiological characteristics of winter bees resemble those of nurse bees, and these winter bee characteristics are already found in bees reared early in the fall.
20. Huang ZY, Robinson GE: *Seasonal changes in juvenile hormone titers and rates of biosynthesis in honey bees*. *J Comp Physiol B* 1995, **165**:18-28.
21. Cherednikov AV: *Photoperiodism in the honey bee, Apis mellifera*. *Entomol Rev* 1967, **46**:33-37.
22. Fluri P, Bogdanov S: *Age dependence of fat body protein in summer and winter bees (Apis mellifera)*. In *Chemistry and Biology of Social Insects*. Edited by Eder J, Rembold H. Verlag J. Peperny; 1987:170-171.
23. Jeffree EP: *Winter brood and pollen in honey bee colonies*. *Insectes Soc* 1956, **3**:417-422.
24. Mattila HR, Otis GW: *Dwindling pollen resources trigger the transition to broodless populations of long-lived honeybees each autumn*. *Ecol Entomol* 2007, **32**:496-505. This study demonstrated that timing of transition from summer bees to winter bees can be influenced by pollen availability.
25. Mattila HR, Otis GW: *Influence of pollen diet in spring on development of honey bee (Hymenoptera: Apidae) colonies*. *J Econ Entomol* 2006, **99**:604-613. This study demonstrated the importance of protein supply and the negative effect of limited protein on the rapid increase colony size of honey bees in spring.
26. Haydak MH: *Honey bee nutrition*. *Annu Rev Entomol* 1970, **15**:143-156.
27. Brodschneider R, Crailsheim K: *Nutrition and health in honey bees*. *Apidologie* 2010, **41**:278-294.

28. Brouwers EVM: **Activation of the hypopharyngeal glands of honeybees in winter.** *J Apic Res* 1983, **22**:137-141.
29. Huang ZY, Otis GW: **Factors determining hypopharyngeal gland activity of worker honey bees (*Apis mellifera* L.).** *Insectes Soc* 1989, **36**:264-276.
30. Maurizio A: **Pollenernahrung und ILcbrnsvorgange bei der Honigbiene.** *Landw Jb Schweiz* 1954:115-182.
31. Bühler A, Lanzrein B, Wille H: **Influence of temperature and carbon dioxide concentration on juvenile hormone titre and dependent parameters of adult worker honey bees (*Apis mellifera* L.).** *J Insect Physiol* 1983, **29**:885-893.
32. Southwick EE: **Metabolic energy of intact honey bee colonies.** *Comp Biochem Physiol A: Physiol* 1982, **71**:277-281.
33. Bloch G, Toma DP, Robinson GE: **Behavioral rhythmicity, age, division of labor and period expression in the honey bee brain.** *J Biol Rhythms* 2001, **16**:444-456.
34. Dreller C, Page RE, Fondrk MK: **Regulation of pollen foraging in honeybee colonies: effects of young brood, stored pollen, and empty space.** *Behav Ecol Sociobiol* 1999, **45**:227-233.
35. Free JB: **Factors determining the collection of pollen by honeybee foragers.** *Anim Behav* 1967, **15**:134-144.
36. Alaux C, Le Conte Y, Adams HA, Rodriguez-Zas S, Grozinger CM, Sinha S, Robinson GE: **Regulation of brain gene expression in honey bees by brood pheromone.** *Genes Brain Behav* 2009, **8**:309-319.
37. Maisonnasse A, Lenoir JC, Beslay D, Crauser D, Le Conte Y: **E- β -ocimene, a volatile brood pheromone involved in social regulation in the honey bee colony (*Apis mellifera*).** *PLoS ONE* 2010, **5**:e13531.
38. Smedal B, Brynne M, Kreibich CD, Amdam GV: **Brood pheromone suppresses physiology of extreme longevity in honeybees (*Apis mellifera*).** *J Exp Biol* 2009, **212**:3795-3801.
 This study demonstrated that brood pheromone exposure alone can reduce the longevity and reduce 'winter bee' physiological characteristics.
39. Leoncini I, Crauser D, Robinson GE, Le Conte Y: **Worker-worker inhibition of honey bee behavioural development independent of queen and brood.** *Insectes Soc* 2004, **51**:392-394.
40. Leoncini I, Le Conte Y, Costagliola G, Plettner E, Toth AL, Wang M, Huang Z, Becard JM, Crauser D, Slessor KN *et al.*: **Regulation of behavioral maturation by a primer pheromone produced by adult worker honey bees.** *Proc Natl Acad Sci U S A* 2004, **101**:17559-17564.
41. Huang ZY, Robinson GE: **Honeybee colony integration: worker-worker interactions mediate hormonally regulated plasticity in division of labor.** *Proc Natl Acad Sci U S A* 1992, **89**:11726-11729.
42. Huang ZY, Robinson GE: **Regulation of honey bee division of labor by colony age demography.** *Behav Ecol Sociobiol* 1996, **39**:147-158.
43. Steinhauer NA, Rennich K, Wilson ME, Caron DM, Lengerich EJ, Pettis JS, Rose R, Skinner JA, Tarpay DR, Wilkes JT *et al.*: **A national survey of managed honey bee 2012-2013 annual colony losses in the USA: results from the Bee Informed Partnership.** *J Apic Res* 2014, **53**:1-18.
44. Guzmán-Novoa E, Eccles L, Calvete Y, MCGowan J, Kelly PG, Correa-Benítez A: **Varroa destructor is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada.** *Apidologie* 2010, **41**:443-450.
45. Sammataro D, Gerson U, Needham G: **Parasitic mites of honey bees: life history, implications, and impact.** *Annu Rev Entomol* 2000, **45**:519-548.
46. Amdam GV, Hartfelder K, Norberg K, Hagen A, Omholt SW: **Altered physiology in worker honey bees (Hymenoptera: Apidae) infested with the mite *Varroa destructor* (Acari: Varroidae): a factor in colony loss during overwintering?** *J Econ Entomol* 2004, **97**:741-747.
47. Bowen-Walker PL, Gunn A: **The effect of the ectoparasitic mite, *Varroa destructor* on adult worker honeybee (*Apis mellifera*) emergence weights, water, protein, carbohydrate, and lipid levels.** *Entomol Exp Appl* 2001, **101**:207-217.
48. Kovac H, Crailsheim K: **Lifespan of *Apis mellifera carnica* Pollm. infested by *Varroa jacobsoni* Oud. in relation to season and extent of infestation.** *J Apic Res* 1988, **27**:230-238.
49. BIP: **National management survey 2013 and 2014 results.** Reported on the BIP website: beeinformed.org.
50. Schmehl DR, Teal PEA, Frazier JL, Grozinger CM: **Genomic analysis of the interaction between pesticide exposure and nutrition in honey bees (*Apis mellifera*).** *J Insect Physiol* 2014, **71**:177-190.
51. Chen YP, Pettis JS, Corona M, Chen WP, Li CJ, Spivak M, Visscher PK, DeGrandi-Hoffman G, iBoncristiani H, Zhao Y: **Israeli acute paralysis virus: epidemiology, pathogenesis and implications for honey bee health.** *PLoS Pathog* 2014, **10**:e1004261.
52. Gisder S, Aumeier P, Genersch E: **Deformed wing virus: replication and viral load in mites (*Varroa destructor*).** *J Gen Virol* 2009, **90**:463-467.
53. Di Prisco G, Pennacchio F, Caprio E, Boncristiani HF, Evans JD, Chen Y: **Varroa destructor is an effective vector of Israeli acute paralysis virus in the honeybee, *Apis mellifera*.** *J Gen Virol* 2011, **92**:151-155.
54. Yang X, Cox-Foster DL: **Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host immunosuppression and viral amplification.** *Proc Natl Acad Sci U S A* 2005, **102**:7470-7475.
55. Di Prisco G, Cavaliere V, Annoscia D, Varricchio P, Caprio E, Nazzi F, Gargiulo G, Pennacchio F: **Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees.** *Proc Natl Acad Sci U S A* 2013, **110**:18466-18471.
56. Buchler R, Costa C, Hatjina F, Andonov S, Meixner MD, Le Conte Y, Uzunov A, Berg S, Bienkowska M, Bouga M *et al.*: **The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera* L. colonies in Europe.** *J Apic Res* 2014, **53**:205-214.
 This study demonstrated substantial effects of genotype (stock) and environment on colony survival, and demonstrated that, at least in Europe, there appear to be locally adapted stocks that have improved performance.
57. Francis RM, Amiri E, Meixner MD, Kryger P, Gajda A, Andonov S, Uzunov A, Topolska G, Charistos L, Wilde J *et al.*: **Effect of genotype and environment on parasite and pathogen levels in one apiary – a case study.** *J Apic Res* 2014, **53**:230-232.
58. SARE 2014 Annual Report. **A comparison of strength and survivability of honeybee colonies started with convention versus northern-requeened packages.** Project Number: FNE12-756. Farmer/Rancher Project Northeast Region.
59. Free JB, Racey PA: **The effect of the size of honeybee colonies on food consumption, brood rearing and the longevity of the bees during winter.** *Ent Exp Appl* 1968, **11**:241-249.
60. Mattila HR, Otis GW: **Manipulating pollen supply in honey bee colonies during the fall does not affect the performance of winter bees.** *Can Entomol* 2007, **139**:554-563.
 This study demonstrated that pollen influx in fall does not affect colony and growth in the consecutive spring.
61. Sagili RR, Breece CR: **Effects of brood pheromone (SuperBoost) on consumption of protein supplement and growth of honey bee (Hymenoptera: Apidae) colonies during fall in a northern temperate climate.** *J Econ Entomol* 2012, **105**:1134-1138.
62. Lait CG, Borden JH, Kovacs E, Moeri OE, Campbell M, Machial CM: **Treatment with synthetic brood pheromone (SuperBoost) enhances honey production and improves overwintering survival of package honey bee (Hymenoptera: Apidae) colonies.** *J Econ Entomol* 2012, **105**:304-331.
63. Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, vanEngelsdorp D, Pettis JS: **High levels of miticides and**

- agrochemicals in North American apiaries: implications for honey bee health.** *PLoS ONE* 2010, **5**:1-19 e9754.
64. Johnson RM, Ellis MD, Mullin CA, Frazier M: **Pesticides and bee toxicity – U.S.A. *Apidologie* Special Issue Review article.** *Apidologie* 2010, **41**:312-331.
 65. Chauzat MP, Martel AC, Cougoule N, Porta P, Lachaize J, Zeggane S, Aubert M, Carpentier P, Faucon JP: **An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera: Apidae) to monitor pesticide presence in continental France.** *Environ Toxicol Chem* 2011, **30**:103-111.
 66. Thompson HM: **Behavioural effects of pesticides in bees – their potential for use in risk assessment.** *Ecotoxicology* 2003, **12**:317-330.
 67. Desneux N, Decourtaye A, Delpuech JM: **The sublethal effects of pesticides on beneficial arthropods.** *Annu Rev Entomol* 2007, **52**:81-106.
 68. Ciarlo TJ, Mullin CA, Frazier JL, Schmehl DR: **Learning impairment in honey bees caused by agricultural spray adjuvants.** *PLOS ONE* 2012, **7**:e40848.
 69. Oruc HH, Hranitz JM, Sorucu A, Duell M, Cakmak I, Aydin L, Orman A: **Determination of acute oral toxicity of flumethrin in honey bees.** *J Econ Entomol* 2012, **105**:1890-1894.
 70. Garrido PM, Antunez K, Martin M, Porrini MP, Zunino P, Eguaras MJ: **Immune-related gene expression in nurse honey bees (*Apis mellifera*) exposed to synthetic acaricides.** *J Insect Physiol* 2013, **59**:113-119.
 71. Pettis JS: **A scientific note on *Varroa destructor* resistance to coumaphos in the United States.** *Apidologie* 2004, **35**:91-92.
 72. Baxter J, Eischen F, Pettis J, Wilson WT, Shimanuki H: **Detection of fluvalinate resistant *Varroa* mites in US honey bees.** *Am Bee J* 1998, **138**:291.
 73. Le Conte Y, Ellis M, Ritter W: ***Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses?** *Apidologie* 2010, **41**:353-363.
 74. Boecking O, Genersch E: **Varroosis – the ongoing crisis in bee keeping.** *J Verbrauch Lebensm* 2008, **3**:221-228.
 75. Highfield AC, El Nagar A, Mackinder LCM, Noël LM-LJ, Hall MJ, Martin SJ, Schroeder DC: **Deformed wing virus implicated in overwintering honeybee colony losses.** *Appl Environ Microbiol* 2009, **75**:7212-7220.
 76. Allen MD, Jeffree EP: **The influence of stored pollen and of colony size on the brood rearing of honeybees.** *Ann Appl Biol* 1956, **44**:649-656.
 77. Voorhies EC, Todd FE, Galbraith JK: **Economic aspects of the bee industry.** *Univ Calif Coll Agric Bull* 1933, **555**:1-117.
 78. Hepburn HR: **Absconding, migration and swarming.** In *Honeybees of Asia*. Edited by Hepburn HR, Radloff SE. Berlin: Springer; 2011:133-158.
 79. Caron DM: *Honey Bee Biology and Beekeeping*. Kalamazoo, MI: Wicwas Press; 2013, 201.