2020

Defining Pollinator Health: A Holistic Approach Based on Ecological, Genetic, and Physiological Factors

Margarita M. López-Uribe
Pennsylvania State University, University Park, mml64@psu.edu

Vincent A. Ricigliano
USDA-ARS, Baton Rouge, Vincent.ricigliano@usda.gov

Michael Simone-Finstrom
USDA-ARS, Baton Rouge, Michael.SimoneFinstrom@usda.gov

ANNUAL REVIEWS

Annual Review of Animal Biosciences

Defining Pollinator Health: A Holistic Approach Based on Ecological, Genetic, and Physiological Factors

Margarita M. López-Uribe,[1,*] Vincent A. Ricigliano,[2,*] and Michael Simone-Finstrom[2,*]

[1]Department of Entomology, Center for Pollinator Research, Pennsylvania State University, University Park, Pennsylvania 16802, USA; email: mml64@psu.edu

[2]Honey Bee Breeding, Genetics and Physiology Research, USDA-ARS, Baton Rouge, Louisiana 70820, USA; email: Vincent.ricigliano@usda.gov, Michael.SimoneFinstrom@usda.gov

Annu. Rev. Anim. Biosci. 2020. 8:269–94

First published as a Review in Advance on October 16, 2019

The *Annual Review of Animal Biosciences* is online at animal.annualreviews.org

https://doi.org/10.1146/annurev-animal-020518-115045

*These authors contributed equally to this article

Keywords

Apis mellifera, bees, biomarkers for health, parasites, pathogens, ecophysiology

Abstract

Evidence for global bee population declines has catalyzed a rapidly evolving area of research that aims to identify the causal factors and to effectively assess the status of pollinator populations. The term pollinator health emerged through efforts to understand causes of bee decline and colony losses, but it lacks a formal definition. In this review, we propose a definition for pollinator health and synthesize the available literature on the application of standardized biomarkers to assess health at the individual, colony, and population levels. We focus on biomarkers in honey bees, a model species, but extrapolate the potential application of these approaches to monitor the health status of wild bee populations. Biomarker-guided health measures can inform beekeeper management decisions, wild bee conservation efforts, and environmental policies. We conclude by addressing challenges to pollinator health from a One Health perspective that emphasizes the interplay between environmental quality and human, animal, and bee health.

1. INTRODUCTION

Insect pollinators play a critical role in the reproduction of close to 90% of plants across all terrestrial ecosystems (1). The ecological role of pollination is therefore vital for supporting plant biodiversity and all associated organisms in the food chain of life, including the sustainability of agriculture and human food security. In the past couple of decades, the increasing pressure for higher crop productivity has resulted in greater demands for pollination, which has had cascading effects on greater demands for managed pollinators, such as the Western honey bee, *Apis mellifera* (2). However, contrary to the needs of the crop pollination industry, managed honey bee populations in North America and Europe currently experience annual colony losses that are on average twice as high as historically (3, 4). In addition, increasing evidence suggests that wild bees and other pollinators, such as flies, butterflies, and florivorous bats, could be facing similar challenges (5). These losses have been linked to several stressors and have triggered calls for more research, public engagement, and policy actions that can help mitigate and improve pollinator health (6). Despite the widespread use of the term pollinator health in the scientific literature and government mandates, this term has not been formally defined, and it is often loosely applied to different biological levels and to groups of pollinators that have different biologies and susceptibilities to stressors.

Translating improved pollinator health into processes that lead to larger pollinator populations that maximize pollination services is a major goal for agricultural production, the beekeeping industry, conservationists, and land managers working on restoration. Ultimately, efforts to improve pollinator health require monitoring of pollinator populations to determine if there has been a change in their health status and then adjusting modifiable variables to improve it. However, three main challenges exist to achieve this goal. First, pollinator health is not well-defined, and effective means of measuring it in wild and managed pollinators with different life history traits (e.g., social versus solitary) are still in their infancy. Second, assessing biological markers (biomarkers) of health can often require killing individuals, which may be a detriment to the population. Third, although environmental stressors impacting pollinators (bees specifically) have been identified (7), effective strategies to monitor and improve pollinator health have received less attention. The establishment of standardized pollinator health measures could facilitate the following: (*a*) linking the health status of pollinator populations with environmental conditions where they live to facilitate decision making for solutions (mitigation) and (*b*) using bee populations as bioindicators of environmental quality for insects and other animals (including humans). Because of the abundance of rapidly evolving literature devoted to pollinator health, this review targets more recent advances to synthesize current approaches to measure health, understand the utility of health biomarkers as a way to more accurately assess health across levels of biological organization, and identify key stressors impacting pollinators. Because there is more information available on *A. mellifera* than on other bee and pollinator species, the primary focus is on honey bees, but we extend the discussion to assessments of health in other managed and wild bees with social and solitary lifestyles.

2. POLLINATOR HEALTH: A DEFINITION

The term bee health first appeared in the literature in the context of disease and toxicology of honey bees (8). In subsequent years, "bee health" and "pollinator health" expanded taxonomically, and they are now used not only for honey bees but also for other pollinating species. Much of the work on this topic has often been somewhat narrow in its approach, largely focusing on detection of parasites and pathogens. For example, a common factor used to assess honey bee health in managed colonies is the quantification of the ectoparasitic mite *Varroa destructor*, hereafter referred to as *Varroa* (9–11). Even though the level of *Varroa* infestation negatively correlates with honey bee

survival (12), mite levels may not correlate well with colony overwintering survival in all cases, as the level of tolerance to the mite can vary across honey bee populations (13) and environmental conditions (14). For wild bee species, pollinator health has often been assessed at the community level using species richness and abundance to characterize the health status of pollinator communities. Although this is a good proxy to characterize bee populations that are hard to study because of methodological constraints, the use of abundance as a metric of pollinator health is often limited by the lack of historical data that can be used as a reference to compare how current population sizes compare to declining ones (but see 5, 15). Therefore, finding biomarkers that can be used to assess and monitor health can help to implement strategies that mitigate stressors and improve the overall health of both managed and wild pollinators.

Health can be generally defined as the state of well-being that translates into the ability of organisms to acquire, allocate, and utilize energy optimally to increase fitness (16). In this sense, we argue that pollinator health is not merely the absence of disease or the presence of abundant numbers of individuals at a certain point in space and time. We define pollinator health as a state that allows individuals to live longer and/or reproduce more, even in the presence of pathogens, thus providing more ecological services. Therefore, pollinator health should be assessed as a comprehensive multilevel measure of the vigor, resilience, and ecological functionality of pollinating species. Health of pollinators can be measured across species by characterizing individuals, colonies, and populations to effectively assess the vulnerability, adaptability, and resilience of different pollinator species to their environmental context (**Figure 1**). A holistic characterization of pollinator health would involve (*a*) metrics incorporating growth, survival, and reproduction at the individual level and (*b*) colony- and (*c*) population-level aspects that quantify the adaptive capacity and resilience of pollinators to environmental conditions. Because of the critical role of pollinators for the functioning of ecosystems that rely on flowering plants as primary producers,

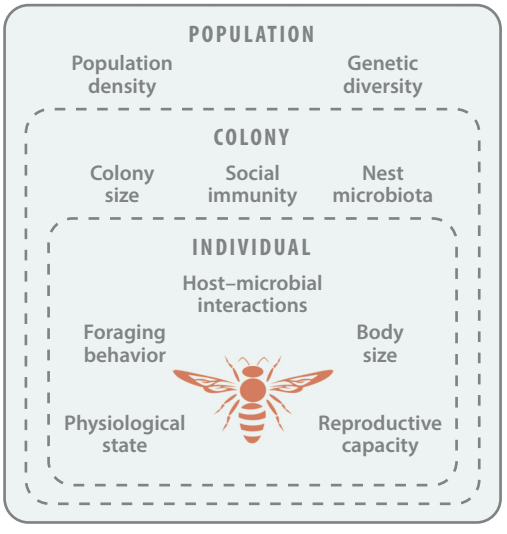

Figure 1

Biological levels of organization relevant for discussion of pollinator health. Inside each box are general ways to assess health status at individual, colony, and population levels. As individual health assessments are often averaged to assess colony health, and colony health is averaged to assess population health, lines surrounding these groups are dashed to represent the flow from the lowest level of biological organization to the higher levels. Figure graphic design by Nick Sloff.

community-level aspects can capture the ability of pollinators to maintain and sustain ecosystem services (17), but these metrics are outside the scope of this review. Here, we discuss an integrative approach to incorporate multiple interacting factors across biological levels of organization. This approach emphasizes a suite of characteristics that are informative across time and changing environmental conditions to best ascertain pollinator health.

3. BIOMARKERS OF POLLINATOR HEALTH

Determination of health occurs at multiple levels of organization (**Figure 1**) and encompasses varying metrics, including genetic, physiological, morphological, and behavioral traits. At the most basic, these metrics can be measures of size (body size, colony size, and population size), productivity (foraging rate, food storage, and reproductive output), and parasite loads. But they can also delve into more nuanced factors, including genetic diversity, microbial community structure, and levels of gene expression. An integration of multiple factors across these levels of biological organization is the most informative approach to assess pollinator health.

The identification and validation of biomarkers that can indicate health status have been a topic of significant research focus in the natural and clinical sciences (18). Applied to pollinators, biomarkers could be used as unbiased differential indicators of declining health status, facilitate the classification and staging of diseased or nondiseased states, and provide quantitative means of assessing nutritional status and physiological responses to the environment. Further, biomarkers can be used to inform management or conservation strategies that can ultimately mitigate colony losses and pollinator declines. Below, we summarize important advancements in the development of biomarkers of honey bee health at the individual and colony levels. These biomarkers can also be used to characterize population health (19), which provides an assessment of the average health status of a group of individuals that live in the same geographic area.

3.1. Individual

Accurately assessing individual health offers baseline information to characterize how environmental stressors may impact the reproductive output and longevity of a species, and how changes in these attributes may impact the biological performance of individuals. Robustness, physiology, microbiota, etc., are different measures indicative of health and ultimately individual fitness.

3.1.1. Measures of individual robustness. At the most basic level, a healthy individual could likely reproduce more effectively, perform its tasks more efficiently, and live longer than an unhealthy one. From the individual reproductive standpoint of social bee species, the egg-laying rate of queens is a strong metric indicative of colony health and has been documented as a marker of sublethal pesticide exposure in honey bees (20) and bumble bees (21). Brood pattern—defined as the number of contiguous cells in an area of comb containing developing larvae or pupae—which has often been a measure of queen quality in honey bees, has recently been identified as a colony phenotype and not a direct measure of queen health (22), perhaps in part due to queen–worker interactions (23). Another predictor of queen health is body size and weight (24). Larger queens tend to mate with more drones, which improves colony health and fitness. Because queens are the reproductive individuals in the colony, research regarding queen size tends to focus on implications for colony health and productivity rather than being queen specific. A heavier queen produces more vitellogenin (VG) (24), a yolk protein that impacts queen fecundity and increases longevity via reducing oxidative stress (25), further suggesting that queen size could play a role in ultimate health and productivity. In worker honey bees, body size is a known indicator of nutritional stress (26) and parasitism (27). However, in *A. mellifera*, body size is more constrained than

NONDESTRUCTIVE BIOMARKERS OF POLLINATOR HEALTH IN WILD BEE POPULATIONS

Biomarkers as discussed for highly populous honey bee colonies can be difficult to use for health assessments of wild bee species, for which determining the locations of their nests is not trivial. In many cases, even when the location of the nests is known, nondestructive methods for sampling have not been developed, hindering the application of biomarkers to determine the health status of individuals without killing them (**Figure 2**). Most studies aiming to characterize the health status of solitary wild bee populations have focused on bee abundance as a proxy of population size and bee health. However, there are caveats with these approaches because of (*a*) the lack of reference for healthy populations and (*b*) the possible variation of population sizes across the distribution of any given species. Despite the limitations of population size as a proxy for health, most other biomarkers (e.g., immune gene expression, lipid content) require sacrificing the individuals to conduct the assays. A promising biomarker for pollinator health of wild solitary bee species is average body size of the population, which can be nonlethally measured in the field from wild-caught individuals. Body size is a variable trait in most bees, and it could be a good indicator of health, as it is correlated with the quality of the food available to forage (29, 35).

in other bees (28), for which body size is largely correlated with the quality of the food available during development. For example, recent studies indicate that bee body size can respond to patterns of landscape simplification, with smaller bees present in highly intensified agricultural fields (29). Therefore, body size could become an important biomarker of health for other managed and wild bee species (see sidebar titled Nondestructive Biomarkers of Pollinator Health in Wild Bee Populations).

Another phenotype that is affected by nutritional and other stressors is foraging efficiency and productivity. Honey bees reared under pollen deprivation not only are smaller but forage at an earlier age and for fewer days and perform inaccurate recruitment behaviors (36). Body size also influences foraging efficiency in bumble bees (37) and is generally predictive of foraging ranges (38). Foraging is not impacted only by nutritional stress, as some pathogen infections also tend to cause similar effects (39–41). With the increased use of passive monitoring tools like radio-frequency identification (RFID) tagging (39, 42), detailed information indicating differences in total foraging life can be determined and provide finer-scale measures of individual bee health, particularly in response to stressors. One measure gained from these tools or from behavioral observations is that age of first foraging is a particularly important characteristic for honey bee health, as typically at the initiation of foraging the rate of mortality increases drastically (43). Age of first foraging, therefore, can be used as a proxy for life span.

3.1.2. Physiological markers of individual health. With precocious foraging in honey bees, biological or physiological age may be decoupled from chronological age, with worker task transitions from brood care to foraging being accelerated (31). Molecular or biochemical biomarkers can be used to directly assess the health statuses of individuals, particularly those related to accelerated aging or accumulation of oxidative stress. Oxidative stress is one way to assess biological age, as accumulation of oxidative damage of lipids, proteins, and DNA impairs functioning and leads to aging (44). How biological age, and not simply chronological age, influences health and resiliency is an important consideration (45, 46). A high accumulation of oxidative damage could be indicative of underlying health concerns (47, 48), but this depends on the current behavioral task of the individual, as foragers, which are more metabolically active, produce more reactive oxygen species and therefore accumulate more damage (49, 50). However,

it also appears that there is a genetic component related to tolerance of oxidative damage (51), which could limit its utility as a biomarker on its own.

Physiological immunity has commonly been used to determine general health. Constitutive immunological defenses (e.g., hemocytes, phenoloxidase cascade) are constantly present and therefore remain relatively static in the background even when individuals or colonies are exposed to pathogens (52). Inducible defenses (e.g., antimicrobial peptides) are those that are activated upon the presence of pathogens or parasites (53). All immune defenses lie somewhere along this gradient, and each can play a crucial role in the overall health of an individual or colony (54). However, it is important to note that both constitutive and inducible immunological defenses can be influenced by biotic and abiotic factors. The use of immune response as a clear biomarker for bee health depends on interpretation and often on other conditions. There are competing assumptions that individuals with a higher level of immune gene expression are under more or constant pathogen pressure (9, 11), or alternatively are more immunocompetent and have access to higher-quality resources and are thus able to invest in immune function more fully (55, 56). However, this does not take into consideration that some individuals and colonies have a higher baseline expression of constitutive immunity (57) or induced responsiveness (58, 59). Furthermore, some pathogens (e.g., deformed wing virus, or DWV) can suppress the honey bee immune system (60, 61), and at some point sick or older individuals may abandon reliance on energetically costly physiological immune responses (62). Looking at immune expression at a static level in individual bees could falsely indicate states of health. However, an individual's ability to mount an immune response, such as the melanization response to inserted nylon threads coated with immune elicitors (61, 63) or immune gene expression after a pathogen challenge (58, 59), may be a clearer indicator of health (30, 58, 63). Assessments of inducible immunity therefore may provide a more direct measure of an individual's ability to combat infection, as constitutive immunity may not always influence disease susceptibility (57). Measuring immune responses at different levels (e.g., enzymatic assays, direct measures of melanization, hemocyte counts, immune gene expression) can provide a robust analysis of immunocompetence (**Figure 2**; **Table 1**).

Laboratory studies have begun to identify other physiological biomarkers of individual bees to assess general health based mainly on nutritional status, such as increased brood food-producing (hypopharyngeal) gland protein content (26); higher abdominal lipid stores (11, 64); higher gene expression for *VG*, a nutritional storage and regulatory protein in worker biology (65, 66); and changes in immune function (55). Smart and colleagues (11) reported one of the first studies to connect individual bee physiological responses to colony health in response to placement landscapes with variable nutritional quality. This study found that at the individual level, *VG* and immune-related gene expression were important markers of overwinter survival. *VG* has emerged as a physiological biomarker because of the role of this protein in resisting oxidative stress (25), response to nutritional resources (11, 65) and parasitism (12, 31), and implications for survival and longevity (25) (**Figure 2**; **Table 1**).

Biomarkers for exposure to pesticides have also been of interest, particularly when a suspected pesticide kill occurs. Esterases and glutathione *S*-transferases (GSTs) have been used widely in other insects but pose some difficulty for assessment in honey bees. Validating the potential use of esterase inhibition and GST levels as biomarkers for pesticide exposure in the laboratory and field has had mixed results (47, 67). Cytochrome *P450* expression is another possible biomarker that has not been fully explored in honey bees, though several studies provide information regarding *P450* expression in response to xenobiotic challenges (68, 69). For each of these enzymatic or gene expression assays, however, different compounds either inhibit or enhance their activity (47, 70). Therefore, interpreting what a certain level of expression means requires at least suggestive evidence of specific exposures. Their use as a biomarker for general pesticide exposure is therefore

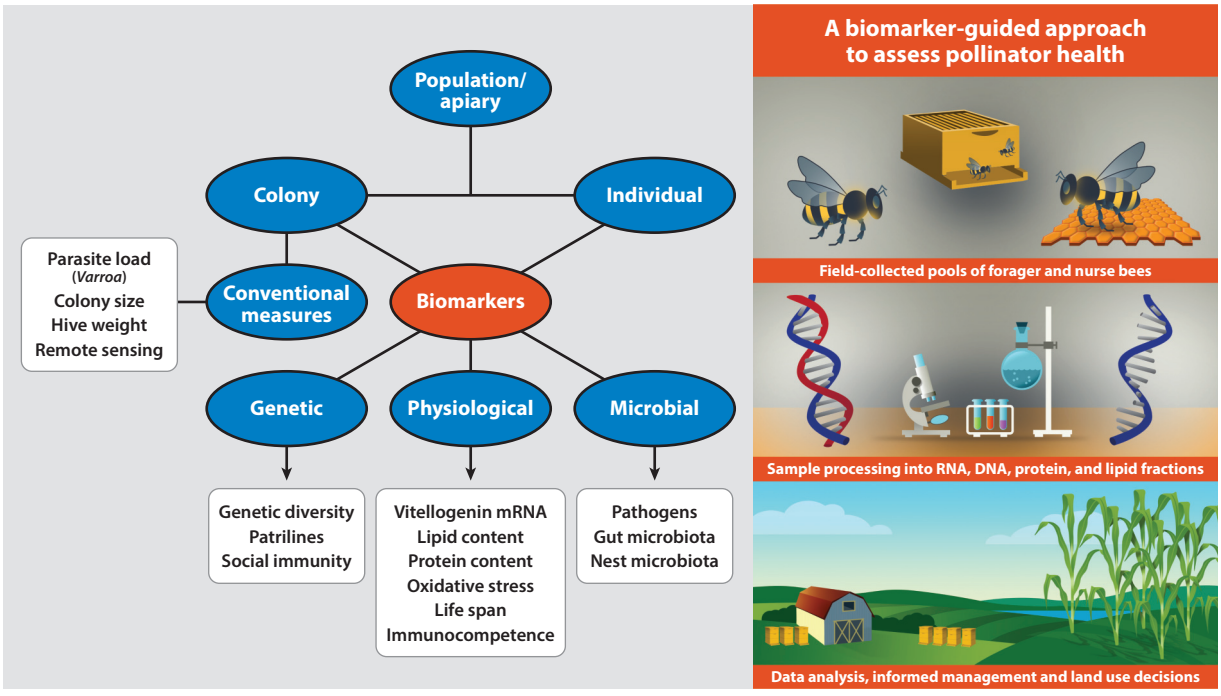

Figure 2

Summary of key genetic, physiological, and microbial biomarkers of honey bees that can be quantified at the individual or colony levels to estimate the average health status of populations. Right panel indicates the three key steps for a biomarker-guided approach to assess pollinator health at different biological levels. Figure graphic design by Nick Sloff.

limited. Lastly, the activity of these enzymes may not actually be correlated to differential sensitivity to pesticides, and model substrates used to assess enzymatic activity may not be good surrogates for pesticide detoxification. Thus, increased levels of enzymatic activity may not be predictive of resiliency to exposure either. Though there is evidence that *P450* expression is related at least to sensitivity to neonicotinoids (69), the utility of biomarkers for pesticide exposure or resilience needs further exploration and validation.

3.1.3. Gut microbiota as an extension of individual health. The microbial communities within insect guts can benefit their hosts by aiding food digestion, synthesizing essential nutrients, detoxifying environmental xenobiotics, competitively excluding pathogens, and modulating host immune functions (71). It has recently been established that social corbiculate bees (honey bees, bumble bees, and stingless bees) share distinct groups of mutualistic and commensal bacteria (i.e., *Snodgrassella*, *Gilliamella*, *Lactobacillus*, and *Bifidobacterium*) (72). In honey bees, the gut microbiota confers nutritive functions such as digestion of recalcitrant pollen components (i.e., hemicelluloses, pectins, and phenolics) (73, 74) and the production of fermentation products (i.e., short chain fatty acids) that contribute to host weight gain (75). Another means by which gut microbiota may influence bee health is by reducing susceptibility to pathogens or parasites. This may be achieved through various mechanisms, including competitive exclusion, alteration of gut physiochemical conditions (76, 77), and immune system stimulation through induction of antimicrobial peptides (78). Altered microbiota structure is associated with impaired host development and increased susceptibility to colonization by pathogens (79). In bumble bees, gut microbiota abundance and

Table 1 Applying integrative approaches to pollinator health: costs and benefits for select measures and biomarkers[a]

Target for assessment	Health measure	Methods	Pros	Cons	References
Parasite and pathogen loads	*Varroa* level	Adult bee washes	Direct, low cost, comparable across populations	Temporal sampling, moderate time investment	92, 93
		Brood infestation	Phenotype for resistance, captures full colony infestation	Time intensive, training required	88–93, 169
	Pathogen abundance, prevalence, and infection intensity	qPCR	Sensitivity, quantitative, pooled sample approach or individuals	Cost, detection of nonreplicating stages, interpretation of relevance of infection levels	132, 135, 170–174
		PCR	Lower cost, pooled or individual samples, can be multiplexed	Sensitivity	170, 171
		Microscopy (e.g., *Nosema*)	Low cost, direct assessment	Sensitivity, specificity, time intensive	175, 176
Colony performance	Bee population	Adult bee assessments	Direct, assessments can be done at different levels of precision (photographically, in-field)	Variability among data collectors, labor intensive, temporally dependent	95
		Brood production and quality	Direct, assessments can be done at different levels of precision (photographically, in-field)	Time and labor intensive, temporal sampling, multiple explanations for poor quality	22, 95
	Nutrient stores	Honey and pollen frame counts	Direct, assessments can be done at different levels of precision (photographically, in-field)	Variability among data collectors, labor intensive, temporally dependent	95
		Colony weight	Direct	Temporally dependent, landscape dependent	95, 177
		Remote sensing of hive metrics and foraging activity	Low labor cost for data collection Fine scale and sensitive data	Length of time to process data, complex interpretations of results, specialized equipment	39, 41, 42, 99, 100, 177
Nutritional status	Lipid storage	Lipid content	Low cost, responsive to nutrition, correlated with immunity and survival, pooled samples	Varies with bee age/task so individual data can be highly variable if not well controlled	11, 64
	Protein production and utilization	*VG* gene expression	Sensitivity, relative quantification, biomarker for aging and health status, pooled samples	Cost	11, 56, 64, 102
		Protein content	Low cost, responsive to nutrition	May vary with bee age/task so individual data can be highly variable if not well controlled	178

(*Continued*)

Table 1 (*Continued*)

Target for assessment	Health measure	Methods	Pros	Cons	References
Immuno-competence	Constitutive immune expression	Enzymatic assays	Lower cost	Sensitivity	62, 179
		Immune gene expression	Sensitivity, relative quantification, pooled samples	Cost	11, 55, 56, 171
	Inducible immune response	Melanization response	Quantitative, direct measure	Time intensive	30, 180
		Immune gene expression	Sensitivity, relative quantification, more direct measure of pathogen susceptibility	Time and labor intensive	48, 59, 181
Microbiota	Microbiota diversity	Genomic sequencing	Fine scale and sensitive data	High cost, technical expertise, direct interpretation for bee health needs validation	85
	Microbiota abundance	Culture-based approaches	Low cost	Sensitivity, labor, technical expertise	182
		qPCR	Sensitivity, relative quantification, diagnostic to determine dysbiosis, pooled samples	Cost	72, 183
		Genomic sequencing	Fine scale and sensitive data	High cost, technical expertise, direct interpretation for bee health needs validation	184
Genetic diversity	Intracolonial diversity	Patriline determination	Comparable across populations	Cost, technical expertise	23, 58, 108, 171
	Population diversity	Allelic variation	Population-level assessment	Cost, technical expertise	143
	Social immunity	Proteomic markers for hygienic behavior	Currently most informative marker-based test for social immunity	Cost, technical expertise, needs further validation	118, 185

[a]The purpose of this table is to highlight integrative measures of pollinator health that can be used for assessments across biological levels of organization. Comments regarding considerations for the use of each metric are included as pros and cons.

Abbreviations: PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; *VG*, vitellogenin.

diversity influence susceptibility to the parasite *Crithidia bombi* (80). Furthermore, intercolony microbiota transplant experiments showed variable capacities to resist *Crithidia* strains, indicating that bacterial strain diversity, and to a lesser extent host–pathogen genetic interactions, can influence immune phenotypes and consequent health outcomes (81). Recent deep sequencing and large-scale culture-dependent analyses have identified extensive strain-level variation within the relatively few bacterial phylotypes associated with honey bees and bumble bees (82, 83). This variation among bacterial strains might confer certain host fitness advantages across different environments (84, 85). In summary, gut microbiota abundance and diversity could be used as biomarkers of bee health and disease susceptibility; however, the functional significance of specific microbes and community compositions is still under investigation.

3.2. Colony

From an agricultural, economic, and evolutionary perspective, the colony is the most important level of organization for eusocial insects. Therefore, a common research goal when working with these insects is to effectively distill colony-level health factors into metrics that accurately reflect survival and performance outcomes under various environmental conditions. Many of these metrics, for honey bees or other species, are an average of individual measures. Colony-specific measures and methods to translate more typical individual-based measures to the colony level are discussed below.

3.2.1. Hive measures associated with colony health. Several measurements can be taken at the colony level as indicators of colony health that can predict the successful overwintering of honey bees. The biggest predictor of honey bee colony survival over the past few decades has been the level of *Varroa* mites, at least when colonies are left untreated (4, 9, 10, 86). Over the years, the level of mites associated with colony losses have decreased substantially, as indicated by late-summer and early-fall treatment thresholds shifting from ~10 mites per 100 adult bees in the late 1990s (87) to only 3 mites per 100 bees by 2010 (3). When *Varroa* loads are managed, colony health is not as impacted by this parasite (56, 64). Thus, monitoring *Varroa* infestation is an important metric of colony health (**Figure 2**; **Table 1**), but *Varroa* numbers can depend on resistance or tolerance traits as well. Therefore, measuring mite population growth from early to late in the season is important, as mite population exponentially increases as colony size and brood rearing ramp up. A lack of mite population growth would be indicative of resistant phenotypes (13, 88, 89), which appears to be related to reducing mite reproduction in brood cells (88, 90, 91). Although monitoring of percent of mites on adult bees is a straightforward test and can be compared across populations (92, 93), infestation in brood where the mites reproduce is a valuable piece of information that is often neglected (93). For non-managed honey bees, an assessment of *Varroa* levels on foraging bees is possible when entrances can be identified and accessed and is indicative of colony health (94) (see sidebar titled Health Biomarkers in Feral Honey Bee Populations).

Various measures of colony strength and productivity are routinely collected to estimate overall colony health, often in response to a treatment or environmental conditions (95–97) (**Figure 2**; **Table 1**). Metrics related to colony size, specifically adult population and amount of brood, are typically strong indicators of colony health (95). Reproductive output in general is highly indicative of health at the colony level; thus, drone production should also be considered (98).

Measures of colony size are often correlated to honey and food storage. From the perspective of beekeepers, managing and selecting breeders based on colony size and honey production are typically the most significant factors of health and ultimately colony fitness. More generally, because foraging for resources is energetically demanding and in temperate climates honey bees

HEALTH BIOMARKERS IN FERAL HONEY BEE POPULATIONS

The development of biomarkers for honey bee health has been facilitated by the comparatively easy assessment of colony status and collection of individuals in Langstroth hive boxes. However, these biomarkers (**Figure 2**) can be difficult to apply for health assessments of feral honey bee colonies—defined as unmanaged honey bees that live in wild conditions—that cannot be easily inspected because they usually nest in closed cavities. Therefore, biomarkers such as mite loads, brood production, size of the adult population, honey production, and social immunity behaviors can be difficult to assess. However, the intensity of mite-transmitted honey bee viruses can serve as an indirect metric for mite loads in the wild honey bee colony (10). Additionally, foragers can be collected to quantify forager immune gene expression, lipid content, oxidative stress, and esterases to estimate an average phenotype for the health status of the colony (30). One of the remaining challenges for studying wild honey bee colonies is to control the role of age when analyzing foragers as a proxy for colony health (31).

need sufficient honey stored for overwintering, honey production is one piece of an integrative assessment of colony health. However, it is important to note that honey storage has a strong genetic component and is also environmentally dependent due to varying resource availability across landscapes. Honey production can be measured by weighing the amount of honey produced over a two-week period of a nectar flow or at the end of the season prior to harvest. With the now more common adoption of remote monitoring techniques, it is also possible to use a hive scale to continuously monitor hive weight. This abundance of real-time information provides not only long-term productivity data but also subtler measures, including changes in forager activity, that can indicate colony-level health perturbations (99) (**Figure 2**; **Table 1**). Remote monitoring can also be used to assess thermoregulatory abilities at the colony level (100). The ability of colonies to efficiently and effectively thermoregulate impacts bee development and colony-level disease outbreaks and so may have clear implications for colony health. Recent work has indicated that thermoregulatory stability can be altered by pesticide exposure and is an informative aspect of colony phenology (99, 100). As mentioned previously, other monitoring methods, such as RFID tagging foragers, can provide detailed information on how foraging behaviors lead to health outcomes at the colony level. Similarly, determining life span via caged studies (101) can provide further insight into identifying subtler treatment effects influencing colony health dynamics (48). Measures of life span are often relevant and undervalued, because perturbations in colony age demographics resulting from an increase in precocious foragers (e.g., in response to pathogens) or sudden loss of a large forager force can impact colony health and reduce colony survival (41, 42).

3.2.2. Physiological markers of colony health. Physiological measures of individual adult honey bees can vary dramatically based on age, nutritional status, seasonal colony demography, and pathogen loads. An emerging method of high-throughput colony-level health assessment employs a pooled field-sampling to overcome individual variation resulting from the spatially constrained, age-based division of labor within a colony (**Figure 2**; **Table 1**). Using separate pools of 50 nurse bees collected from the center of the brood nest or 25 forager bees vacuum aspirated off the entrance of the hive, Ricigliano and colleagues (102) reported colony-level gene expression and pathogen levels that reflected traditional colony metrics such as brood production, food stores, and *Varroa* infestation. Notably, expression analyses of *VG* and recently identified *VG*-like gene homologs (103) in nurse bees were positively correlated with colony performance, phenology, and immune-related gene transcript levels. Using the same methodology, Ricigliano and colleagues (56) reported that apiaries within foraging distance of reclaimed prairies (as part

of the US Conservation Reserve Program lands) had significantly improved performance, better overwinter survival potential, and higher colony-level expression of *VG* and immune-related genes. The study also found that pre- and post-winter *VG* levels were significantly correlated with adult bee mass by analyzing colony weight data. Similarly, Alaux and colleagues (64) analyzed *VG* in pools of 30 abdomens and noted that it was also a strong predictor of overwintering survival, as previously reported using an individual-bee approach (11). *VG* has further been described, again using pooled samples, as a way to assess demographic changes that could be indicative of colony failures or health issues (31). This approach, based on quantification of gene expression, has also been used recently to examine markers of immune health in response to infection status, showing high correlations between immune gene expression, colony-level *Varroa* loads, and viral infection (104). It is worth noting that if particular biomarkers of immune health are simply correlated to infection status, then measuring infection intensity or prevalence may be sufficient and cost-saving, since physical measures can be used to assess at least some pathogen and parasite loads (e.g., *Varroa* infestation or *Nosema* infection).

Analyses of averaged individual bee samples can lead to differential results as compared with pooled samples for some physiological measures. For example, Smart and colleagues (11) found that expression of immune-related genes *defensin1* and *lysozyme2* for averaged individual bees of known age was negatively correlated with colony survival and *VG* levels, whereas another study conducted in the same geographic area used a pooled sampling approach and determined that immune gene expression largely reflected *VG* levels and colony performance (56). A pooling approach can better capture the whole colony response, whereas individual bee analyses are highly dependent on both chronological and biological age and can lead to high variation. However, two pools per colony may be needed if the research questions aim to capture variation in the health status of individuals based on tasks (i.e., nurse bees versus active foragers) (102). This is particularly true for measures of oxidative stress, for which there can be extreme variation in individuals based on biological age, chronological age, task, and tolerance (48, 50, 51). Therefore, conducting analyses with pooled samples can reduce the ability to detect differences across treatments or landscapes (M. Simone-Finstrom & V.A. Ricigliano, personal observations). Currently the standard is to conduct measures of oxidative stress at the individual level; measurement of oxidative damage in pooled samples still requires validation. Nevertheless, these results highlight the potential of pooled sample molecular and biochemical diagnostics to obtain colony-level health information in response to landscape variation.

3.2.3. Genetic determinants of colony health and resilience. Genetic differences drive much of the variation in differential impacts that stressors have on colony health (14). Much interest has focused on how genetic diversity can impact colony-level health and productivity (98, 105). High intracolonial genetic diversity—determined by the number of patrilines within a colony based on the number of males the queen mates with—has been demonstrated to reduce disease intensity and prevalence (106, 107) and increase honey bee colony survival in commercial beekeeping operations (108). Genetic variation within a colony helps to maintain diverse alleles that contribute to differential susceptibility to various stressors and can increase a colony's ability to withstand varying environments and emerging threats. Honey bees are extreme in that a queen will mate with 10–30 drones. Multiple mating in Hymenoptera is rare (109); thus, high intracolonial genetic diversity is a major factor advantage only with respect to honey bees.

Genetics also specifically influences expression of disease resistance traits. Although the use of immune responses as metrics of health has largely relied on the individual immune response, social insects also have a colony-level or social immune response composed of traits that reduce pathogen and parasite infection intensity and transmission at the colony level (110–112). Recent

iterations of the definition of social immunity have been expanded to include any defense against parasites and pathogens that evolved and is maintained to benefit group members. Thus, behaviors associated with parental care are part of social immunity because they are critical for colony health and development (113). Mechanisms of social immunity include grooming of nestmates, incorporation of glandular secretions in the nest environment, collection and use of plant-produced antimicrobials, and hygienic behavior (111)—that involves the detection and removal of sick larvae or prepupae before they become infectious, thus reducing the spread of disease at the colony level (112). A more specific type of hygienic behavior, *Varroa* sensitive hygiene (VSH), specifically targets pupal cells infested by reproductive *Varroa*, functionally reducing the mite population growth in a colony (88, 90, 91), while subsequently also likely preventing the spread of viruses vectored by mite infestation (39). Hygienic behavior is a trait that can be measured using standardized assays (112), though colony-level expression of the trait can vary somewhat based on environmental conditions, including high nectar flows (114, 115), pesticide exposure (20, 116), and potentially even viral infection (117). In these cases, multiple assessments of hygienic behavior are necessary because a low (or high) expression of the trait could be more influenced by environmental conditions rather than genetics.

From a biomarker perspective, proteomic markers have been identified and used in a successful breeding program for colonies with a high level of hygienic behavior (118). A 13-protein marker panel was established based on assessments from single pools of 10 or 30 worker antennae, again highlighting the value of pooled sampling approaches to detect major colony phenotypes (**Table 1**). Genomic markers for hygienic behavior and VSH have also been of significant interest (119), though this approach has not yet led to marker-assisted selection. The identification of genetic, proteomic, or kinomic markers associated with traits of interest, particularly with respect to social immunity, would ultimately allow for more targeted multi-trait selection in breeding populations. For a greater understanding of colony health, these markers could also be used to establish a social immunity index that would be indicative of a colony's ability to withstand various stressors. Colonies with a larger social immunity tool kit would be predicted to be more resilient or immunocompetent at the colony level. This index could be developed by ranking colony performance for each social immune trait and developing a total social immunity score (similar to 120). A more complex index could be developed weighting the potential influence of each of these different traits in response to environmental conditions (e.g., 121).

3.2.4. Connecting individual physiology and social immune responses. As social immune responses are predicated by the coordinated behaviors of individuals, expression of social immunity depends on the physiological states of individuals. This interaction between individual physiology and social immunity and how they both ultimately influence colony health is quite complex. For example, a physiological immune response in some workers can induce grooming behavior in nestmates (122). Similarly, larval signaling can trigger an immune response and may cause hygienic removal of sick individuals (123). In contrast, the collection of antimicrobial plant resins to produce propolis that is added to the honey bee hive environment allows individual honey bees to relax investment in immune function (reviewed in 124). Here a social immune trait is influencing individual bee physiology. Further, honey bees have been shown to socially medicate in response to various pathogens and parasites, but the mechanisms activating these responses are currently unclear. It is possible that individual physiological responses to challenges induce a self- or social medication response or that the response is the result of signals given by nestmates (125). Understanding the mechanisms influencing the induction of the different social immune defenses would shed light on how they can be measured more effectively as part of a social immunity index.

Interactions between individual and social immune defenses become a bit more complicated when physiological traits are co-opted as a mechanism of social immunity, such as the use of venom as an antimicrobial (126) or the use of glucose oxidase (GOX) in larval diet and honey stores (127). GOX is produced by older nectar-processing honey bees, and it may be co-opted by nurse bees to serve as an antimicrobial in brood food. GOX is also responsible for a major portion of the antimicrobial activity of honey and somewhat for royal jelly. This interplay between individuals producing GOX and its function as a social immune defense complicates its utility as a biomarker for colony health and investment in social immunity (54), despite its growing use as such (55, 128). GOX expression clearly varies across colonies (54, 127), which could be due to environmental or genetic factors. GOX requires further validation for its role as a biomarker of social immunity.

3.2.5. Microbial markers as an assessment of the extended colony health phenotype. Since the occurrence of colony collapse disorder (CCD) in 2006, much focus has been placed on identifying the pathogens most associated with colony losses (129–131). While this process ultimately led to the conclusion that colony losses are the result of several interacting stressors, the specifics can differ from case to case. The massive number of colonies lost after the appearance of CCD also led to research efforts to generate information about pathogen loads across pollinator communities, allowing the identification of possible emerging threats to bee health (132, 133). Common viruses vectored by *Varroa* were identified as being associated with colony loss, particularly DWV, and have been found in non-*Apis* bees (9, 10, 86). As a marker of colony loss, however, these viral levels are typically measured pre-winter, and so from a management perspective, by the time the analysis is done it may be too late to take corrective action. DWV titers closely track *Varroa* loads (peaking in late summer or early fall), whereas other viral infections and other pathogens and parasites can have different temporal dynamics.

A common approach to assess total pathogen load is to sample colonies over time (134) and total the number of pathogenic microbes detected in a colony throughout a season to account for the seasonality of particular pathogens. This discrete value can be further refined by totaling just the number of pathogens detected above a particular threshold to indicate only severe infections (86). It does appear that higher pathogen loads leading to co-infections tend to be associated with colony failures, as many pathogens may be more opportunistic. This holistic measure of colony-level infection should not be based just on viral infection but should include the larger suite of pathogens and parasites that can attack honey bee colonies (e.g., *Nosema*, European foulbrood, American foulbrood, chalkbrood, trypanosomes). Based on this concept, pathogen prevalence within a colony can be a useful metric of colony health (**Figure 2**; **Table 1**). However, for accurate measures of pathogen prevalence for each specific microbe, bees need to be analyzed individually, as described elsewhere (134, 135). If a single pool is used per colony, then the measure would simply indicate pathogen abundance. Limits of detection for pathogens based on pool size have been mathematically calculated, suggesting that if only 5% of the bees in a colony were infected with a particular pathogen, a pool of 59 bees would be required to detect that infection with a 95% probability (135). This is probably more than sufficient for any biologically meaningful infection. It is important to consider, however, that pathogen load can interact with bee genotype and may be misleading if mechanisms like tolerance are at play (136) or may lead to a false indicator of health if colonies or individuals are highly susceptible to low-level infections (e.g., 137).

Commensal microbes can be considered as part of the extended colony health phenotype. Whereas gut microbiota has been a major focus of study, the influence of nonpathogenic nest bacteria on bee health is comparatively less known. In social bees, bacterial niches exist within individuals, groups, and the nest. In contrast, solitary bees have a different lifestyle in which there

is no mouth-to-mouth contact among adults, restricting pathogen transmission to food provisions. Whereas specific groups of lactic acid bacteria have coevolved with specific bee lineages, closely related environmental and flower-associated lactobacilli colonize both social and solitary bee nest materials (85, 138). These lactobacilli are found in stored pollen, nectar, and brood provisions, suggesting they play a role in inhibiting spoilage microorganisms and brood pathogens (139, 140).

3.3. Population

Pollinator population status is typically assessed by measures of species abundance that can elucidate the census population sizes of different species across their geographic ranges (141) (**Figure 1**). Although these metrics can be valuable to assess the effects of recent environmental changes on population size, these approaches can capture detrimental effects only in the presence of longitudinal data from monitoring programs or historical collections (e.g., 5, 142). Promising alternative approaches to identify environmental factors influencing managed honey bee population health incorporate longitudinal assessments of colony performance and physiology across variable landscapes (64, 100).

From an evolutionary perspective, the population is the smallest unit that can undergo evolution. Genetic diversity at the population level is a metric of evolutionary potential and key to resilience against changing stressors, environmental conditions, and emerging parasites and pathogens (143; see sidebar titled Genetic Biomarkers of Pollinator Health in Social Wild Bee Populations). In general, large-bodied social bees are characterized by populations with high levels of genetic diversity and low spatial genetic structure, meaning that individuals come from the same genetic pool over large geographic distances (144, 145). However, small- to mid-bodied solitary bees, approximately 70% of all bee species, can have lower levels of genetic diversity distributed in highly genetically structured populations, resulting in levels of genetic diversity that can vary significantly across small geographic scales. For honey bees, an apiary can be considered a population, because a single apiary houses a group of colonies exposed to a specific landscape and microclimate conditions that make it distinct from even nearby apiaries. Therefore, population health measures for managed honey bees can be derived from spatially constrained averages of colony health at the scale of an apiary. Characterizing patterns of genetic diversity at the population level can also be

GENETIC BIOMARKERS OF POLLINATOR HEALTH IN SOCIAL WILD BEE POPULATIONS

Biomarkers to establish population-level health metrics are particularly informative for understanding the stability and health of wild bees. For wild social bees, foragers can be sampled to characterize the mean phenotype of a colony without destroying the colony after sampling. However, in many cases, locating the nests of wild bee species can be difficult. Using population genetic approaches to estimate family relationships in wild-caught individuals offers the possibility to determine the mean health phenotype at the population level while correcting for the family structure of social insects. In brief, wild-caught individuals are profiled via genetic markers (e.g., microsatellites, single-nucleotide polymorphisms) and assigned to family groups through the estimation of relatedness. There are multiple methodological approaches to estimate genetic relatedness (32). These same genetic approaches can also be used to estimate colony density as a proxy for the number of reproductive units of social bees that are present in any given landscape (33). Methods developed for nonlethal sampling of wild-caught social bees facilitate assessment of biomarkers of health (34).

used to detect population bottlenecks (146) or population expansions (147). However, the utility of genetic markers to detect recent demographic changes in ecological time remains to be tested.

4. INTERACTING STRESSORS IMPACTING POLLINATOR HEALTH

Growing evidence indicates that threats to the long-term stability of bee populations are related to interactions between abiotic and biotic stressors. The conversion of 40% of the land on terrestrial ecosystems for agriculture and pastures has had several cascading effects on pollinators. The dominance of agricultural landscapes has reduced the total diversity—both abundance and richness—of available floral resources (148). This translates into poor nutritional landscapes for bees. Even though floral resources can increase temporarily during crop bloom and agricultural weeds can subsidize bee populations, the overall quality of these floral resources may not be optimal (149, 150). Bees require specific ratios of proteins, lipids, and carbohydrates in their diets (26), meaning that bees need to forage on diverse sources to support individual and colony health (55, 66).

Poor nutritional landscapes that result from increased agricultural intensity are typically accompanied by increased chemical inputs that contaminate floral resources. Pesticide applications in agricultural ecosystems are a compounding key factor with direct implications for bee health. Pest control through the use of pesticides—whether conventional or organic—is necessary within most production systems to protect crops from economically significant losses and to maintain farm viability. However, many commonly used agrochemicals have lethal and sublethal effects on managed and wild bees (151, 152). Human-applied insecticides, miticides, fungicides, and antibiotics each have different proposed mechanisms by which they impact bee health (68). Neonicotinoids, a ubiquitous group of synthetic nicotine derivatives, can have acute toxicities in bees, and their sublethal effects can be delayed or difficult to quantify because they may interact with other stressors (153, 154). A unique chemical stressor managed honey bees face is the application of miticides to control *Varroa*. Miticides are applied inside the hive and lead to individuals under constant exposure for the duration of the treatment. Miticides or their related transformation products are some of the most prevalent compounds detected in bee hives (155) and can negatively impact nutritional, immunological, and developmental biomarkers (156). Fungicides are another class of compounds detected in bee colonies and appear to exert most of their effects on brood (157), whereas antibiotic exposure decreases the abundance and diversity of beneficial microbiota in adults, which can negatively impact host health (158). Increasing fungicide use has also been directly linked to declines and range contractions of bumble bees in North America (159).

Honey bee pathogens have been intensively studied and currently include a broad spectrum of pathogenic microbes (the pathosphere), which challenge bees in a multitude of combinations with other stressors (130). Interactions between bee genetics and the commensal microbiota (together considered the hologenome) likely mediate the impact of the pathosphere on host health (131). A meta-analysis of multiple surveys of pathogen incidence and/or abundance in relation to colony survival did not reveal a single pathogen that was consistently correlated with colony loss (131). Instead, this study identified pathogens that were frequently found in all colonies, including several viruses, *Nosema*, and trypanosomatids. This is consistent with the current paradigm that bee health is currently challenged by multiple interacting stressors and that responses at the individual, colony, and population level are context dependent. For honey bees, energetic costs associated with coinfections by various pathogens and constant agrochemical exposure are likely exacerbated in a background of *Varroa* infestation and poor nutrition (160, 161). Biomarker approaches have potential for increased sensitivity to tease apart interacting stressors in ways that are not possible with conventional measures of colony survival and performance (**Figure 2**).

5. A ONE HEALTH APPROACH TO IMPROVE POLLINATOR HEALTH

Although the development of biomarkers can facilitate monitoring programs for pollinator health, effective solutions to mitigate stressors will be hard to promote and implement unless they are placed in a more holistic context, like a One Health logic. The concept of One Health links animal health, environmental quality, and human health outcomes, both conceptually and in practice (162). There are multiple ways in which pollinator health can be linked to the health of humans, other animals, and the environment. Healthy bee populations and diverse communities contribute to stable ecosystem services that are directly linked to human health through the production of more diverse and nutritious foods (163, 164). Landscapes that provide more foraging habitats with lower chemical inputs can support larger populations of other beneficial insects, which can in turn increase ecosystem services such as biological control (165). On the other hand, increasing pollinator habitat in human-dominated landscapes, such as urban areas, could have indirect benefits to humans. For example, it has been demonstrated that the presence of more green areas in cities is linked to reduced anger and anxiety in humans. In summary, improving pollinator health can lead to increasing food security, human health, biodiversity, and ecological services. Improving pollinator health should be valued as part of an effort to maintain and sustain ecosystem integrity, which will confer comprehensive well-being to humans and other animals (166). Fully understanding the range of potential benefits of the One Health approach for improving pollinator health requires a deeper understanding of how humans, animals, pollinators, and their environment are interrelated.

Environmental quality has a key role in maintaining and restoring pollinator health status. Higher-quality habitats can be classified as those that can support healthier populations with longer life spans for bee individuals, colonies, and populations. High-quality environments for bees are determined by several components. The first is the availability of floral resources that can provide nutritious diets for larval development, foraging, nest construction, and immune function (35, 150). Nutritional needs vary widely among bee species, from narrow specialists (oligolectic species) that use only a restricted number of plants to highly generalist species that can consume pollen from most plant species (167). Therefore, the composition of high-quality nutritional landscapes may vary for oligolectic species that could benefit from monocultures of particular plant species [e.g., *Eucera* (*Peponapis*) *pruinosa* specialists on pumpkin and squash pollen] and for polylectic species that need diverse pollen resources. Second, high-quality landscapes must provide nesting resources for bees, including cavities, wood, twigs, and well-drained soils with a variety of texture characteristics (168). Other resources that bees may need for nest construction include plant resins, oils, mud, and water. Availability of all of these resources, in landscapes free of chemical compounds that are toxic to bees through contact and ingestion, is critical to guarantee a healthy environment.

6. CONCLUSIONS

With recent declines in pollinators at the population level and annual colony losses reaching unsustainable levels, clear assessments of pollinator health and the factors influencing them are of increasing importance. A biomarker-guided approach has the potential to provide high-resolution pollinator health measures in the context of different interacting biotic and abiotic stressors, landscape variations, and management practices. Early identification of factors negatively impacting pollinator health can enable real-time management or policy adjustments before detrimental effects can manifest at the population and community levels. Similarly, the identification of positive health drivers can inform management decisions and conservation efforts at local and regional scales. Although they hold significant promise, biomarkers of pollinator health require further

development to identify sensitive and useful targets, especially across a range of seasonal, geographic, and management scenarios. Information obtained using a biomarker approach has the potential for the most impact if it is incorporated into the multidisciplinary framework of One Health. The resulting changes in human practices could generate positive effects for humans, animals, and pollinators.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors thank Bob Danka, Kate Ilhe, William Meikle, and Frank Rinkevich for critical review of the manuscript. M.M. López-Uribe was supported by the USDA National Institute of Food and Agriculture and Multistate Research Project PEN04579.

LITERATURE CITED

1. Ollerton J, Winfree R, Tarrant S. 2011. How many flowering plants are pollinated by animals? *Oikos* 120:321–26
2. Aizen MA, Harder LD. 2009. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Curr. Biol.* 19:915–18
3. Genersch E, von der Ohe W, Kaatz H, Schroeder A, Otten C, et al. 2010. The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. *Apidologie* 41:332–52
4. Kulhanek K, Steinhauer N, Rennich K, Caron DM, Sagili RR, et al. 2017. A national survey of managed honey bee 2015–2016 annual colony losses in the USA. *J. Apic. Res.* 56:328–40
5. Mathiasson ME, Rehan SM. 2019. Status changes in the wild bees of north-eastern North America over 125 years revealed through museum specimens. *Insect Conserv. Divers.* 12:278–88
6. **Liere H, Jha S, Philpott SM. 2017. Intersection between biodiversity conservation, agroecology, and ecosystem services. *Agroecol. Sustain. Food Syst.* 41:723–60**
7. Goulson D, Nicholls E, Botias C, Rotheray EL. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347:1255957
8. Hillyard TN. 1965. Facts about beekeeping in England. *Bee World* 46:77–85
9. Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P. 2012. Predictive markers of honey bee colony collapse. *PLOS ONE* 7:e32151
10. Kielmanowicz MG, Inberg A, Lerner IM, Golani Y, Brown N, et al. 2015. Prospective large-scale field study generates predictive model identifying major contributors to colony losses. *PLOS Pathog.* 11:e1004816
11. **Smart M, Pettis J, Rice N, Browning Z, Spivak M. 2016. Linking measures of colony and individual honey bee health to survival among apiaries exposed to varying agricultural land use. *PLOS ONE* 11:e0152685**
12. Amdam GV, Hartfelder K, Norberg K, Hagen A, Omholt SW. 2004. 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.* 97:741–47
13. Locke B. 2016. Natural *Varroa* mite-surviving *Apis mellifera* honeybee populations. *Apidologie* 47:467–82
14. Meixner MD, Kryger P, Costa C. 2015. Effects of genotype, environment, and their interactions on honey bee health in Europe. *Curr. Opin. Insect Sci.* 10:177–84
15. Wood TJ, Gibbs J, Graham KK, Isaacs R. 2019. Narrow pollen diets are associated with declining Midwestern bumble bee species. *Ecology* 100:e02697
16. Bortz WM. 2005. Biological basis of determinants of health. *Am. J. Public Health* 95:389–92

6. Review highlights the close relationship between agricultural management practices, biodiversity, landscape composition, and their potential effects on pollination services.

11. First study connecting effects of agricultural intensity to colony performance and individual bee health.

17. Winfree R, Reilly JR, Bartomeus I, Cariveau DP, Williams NM, Gibbs J. 2018. Species turnover promotes the importance of bee diversity for crop pollination at regional scales. *Science* 359:791–93

18. Ptolemy AS, Rifai N. 2010. What is a biomarker? Research investments and lack of clinical integration necessitate a review of biomarker terminology and validation schema. *Scand. J. Clin. Lab. Investig.* 70:6–14
19. Kindig D, Stoddart G. 2003. What is population health? *Am. J. Public Health* 93:380–83
20. Wu-Smart J, Spivak M. 2016. Sub-lethal effects of dietary neonicotinoid insecticide exposure on honey bee queen fecundity and colony development. *Sci. Rep.* 6:32108
21. Wu-Smart J, Spivak M. 2018. Effects of neonicotinoid imidacloprid exposure on bumble bee (Hymenoptera: Apidae) queen survival and nest initiation. *Environ. Entomol.* 47:55–62

22. Lee KV, Goblirsch M, McDermott E, Tarpy DR, Spivak M. 2019. Is the brood pattern within a honey bee colony a reliable indicator of queen quality? *Insects* 10:12

23. Fine JD, Shpigler HY, Ray AM, Beach NJ, Sankey AL, et al. 2018. Quantifying the effects of pollen nutrition on honey bee queen egg laying with a new laboratory system. *PLOS ONE* 13(9):e0203444. **https://doi.org/10.1371/journal.pone.0203444**
24. Delaney DA, Keller JJ, Caren JR, Tarpy DR. 2011. The physical, insemination, and reproductive quality of honey bee queens (*Apis mellifera* L.). *Apidologie* 42:1–13
25. Seehuus SC, Norberg K, Gimsa U, Krekling T, Amdam GV. 2006. Reproductive protein protects functionally sterile honey bee workers from oxidative stress. *PNAS* 103:962–67
26. Brodschneider R, Crailsheim K. 2010. Nutrition and health in honey bees. *Apidologie* 41:278–94
27. Bowen-Walker PL, Gunn A. 2001. 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.* 101:207–17
28. Roulston TH, Cane JH. 2000. The effect of diet breadth and nesting ecology on body size variation in bees (Apiformes). *J. Kans. Entomol. Soc.* 73:129–42

29. Renauld M, Hutchinson A, Loeb G, Poveda K, Connelly H. 2016. Landscape simplification constrains adult size in a native ground-nesting bee. *PLOS ONE* 11:e0150946

30. Youngsteadt E, Appler RH, López-Uribe MM, Tarpy DR, Frank SD. 2015. Urbanization increases pathogen pressure on feral and managed honey bees. *PLOS ONE* 10:e0142031
31. Alaux C, Soubeyrand S, Prado A, Peruzzi M, Maisonnasse A, et al. 2018. Measuring biological age to assess colony demographics in honeybees. *PLOS ONE* 13:e0209192
32. Wang J. 2014. Marker-based estimates of relatedness and inbreeding coefficients: an assessment of current methods. *J. Evol. Biol.* 27:518–30
33. Jha S, Kremen C. 2013. Resource diversity and landscape-level homogeneity drive native bee foraging. *PNAS* 110:555–58
34. Scriven JJ, Woodall LC, Goulson D. 2013. Nondestructive DNA sampling from bumblebee faeces. *Mol. Ecol. Resourc.* 13:225–29

35. Roulston TH, Goodell K. 2011. The role of resources and risks in regulating wild bee populations. *Annu. Rev. Entomol.* 56:293–312

36. Scofield HN, Mattila HR. 2015. Honey bee workers that are pollen stressed as larvae become poor foragers and waggle dancers as adults. *PLOS ONE* 10:e0121731
37. Goulson D, Peat J, Stout JC, Tucker J, Darvill B, et al. 2002. Can alloethism in workers of the bumblebee, *Bombus terrestris*, be explained in terms of foraging efficiency? *Anim. Behav.* 64:123–30
38. Greenleaf SS, Williams NM, Winfree R, Kremen C. 2007. Bee foraging ranges and their relationship to body size. *Oecologia* 153:589–96
39. de Guzman L, Frake A, Simone-Finstrom M. 2017. Comparative flight activities and pathogen load of two stocks of honey bees reared in gamma-irradiated combs. *Insects* 8:127
40. Goblirsch M, Huang ZY, Spivak M. 2013. Physiological and behavioral changes in honey bees (*Apis mellifera*) induced by *Nosema ceranae* infection. *PLOS ONE* 8:e58165
41. Natsopoulou ME, McMahon DP, Paxton RJ. 2016. Parasites modulate within-colony activity and accelerate the temporal polyethism schedule of a social insect, the honey bee. *Behav. Ecol. Sociobiol.* 70:1019–31

17. This study demonstrates the importance of beta-diversity to maximize pollination services at regional scales.

22. Highlights the complexity of colony phenotype and shows that laying pattern is often misconstrued as only a queen problem.

29. Empirical study that reports significantly smaller body size of individuals in more intensified agricultural areas.

35. Comprehensive review of the different factors regulating population size in wild bees.

42. Perry CJ, Søvik E, Myerscough MR, Barron AB. 2015. Rapid behavioral maturation accelerates failure of stressed honey bee colonies. *PNAS* 112:3427–32
43. Rueppell O, Bachelier C, Fondrk MK, Page RE Jr. 2007. Regulation of life history determines lifespan of worker honey bees (*Apis mellifera* L.). *Exp. Gerontol.* 42:1020–32
44. Terman A, Brunk UT. 2006. Oxidative stress, accumulation of biological "garbage," and aging. *Antioxid. Redox Signal.* 8:197–204
45. Anderson KE, Ricigliano VA, Mott BM, Copeland DC, Floyd AS, Maes P. 2018. The queen's gut refines with age: longevity phenotypes in a social insect model. *Microbiome* 6:108
46. Seehuus SC, Krekling T, Amdam GV. 2006. Cellular senescence in honey bee brain is largely independent of chronological age. *Exp. Gerontol.* 41:1117–25
47. Carvalho SM, Belzunces LP, Carvalho GA, Brunet J-L, Badiou-Beneteau A. 2013. Enzymatic biomarkers as tools to assess environmental quality: a case study of exposure of the honeybee *Apis mellifera* to insecticides. *Environ. Toxicol. Chem.* 32:2117–24
48. Simone-Finstrom M, Li-Byarlay H, Huang MH, Strand MK, Rueppell O, Tarpy DR. 2016. Migratory management and environmental conditions affect lifespan and oxidative stress in honey bees. *Sci. Rep.* 6:32023
49. Margotta JW, Roberts SP, Elekonich MM. 2018. Effects of flight activity and age on oxidative damage in the honey bee, *Apis mellifera*. *J. Exp. Biol.* 221:jeb183228
50. Williams JB, Roberts SP, Elekonich MM. 2008. Age and natural metabolically-intensive behavior affect oxidative stress and antioxidant mechanisms. *Exp. Gerontol.* 43:538–49
51. Li-Byarlay H, Huang MH, Simone-Finstrom M, Strand MK, Tarpy DR, Rueppell O. 2016. Honey bee (*Apis mellifera*) drones survive oxidative stress due to increased tolerance instead of avoidance or repair of oxidative damage. *Exp. Gerontol.* 83:15–21
52. Schmid-Hempel P. 2005. Evolutionary ecology of insect immune defenses. *Annu. Rev. Entomol.* 50:529–51
53. Evans JD, Aronstein K, Chen YP, Hetru C, Imler JL, et al. 2006. Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Mol. Biol.* 15:645–56
54. López-Uribe MM, Fitzgerald A, Simone-Finstrom M. 2017. Inducible versus constitutive social immunity: examining effects of colony infection on glucose oxidase and defensin-1 production in honeybees. *R. Soc. Open Sci.* 4:170224
55. Alaux C, Ducloz F, Crauser D, Le Conte Y. 2010. Diet effects on honeybee immunocompetence. *Biol. Lett.* 6:562–65
56. **Ricigliano VA, Mott BM, Maes PW, Floyd AS, Fitz W, et al. 2019. Honey bee colony performance and health are enhanced by apiary proximity to US Conservation Reserve Program (CRP) lands. *Sci. Rep.* 9:4894**
57. Evison SEF, Fazio G, Chappell P, Foley K, Jensen AB, Hughes WOH. 2016. Innate expression of antimicrobial peptides does not explain genotypic diversity in resistance to fungal brood parasites in the honey bee. *Apidologie* 47:206–15
58. Simone-Finstrom M, Walz M, Tarpy DR. 2016. Genetic diversity confers colony-level benefits due to individual immunity. *Biol. Lett.* 12:20151007
59. Decanini LI, Collins AM, Evans JD. 2007. Variation and heritability in immune gene expression by diseased honeybees. *J. Hered.* 98:195–201
60. Nazzi F, Pennacchio F. 2018. Honey bee antiviral immune barriers as affected by multiple stress factors: a novel paradigm to interpret colony health decline and collapse. *Viruses* 10:159
61. Di Prisco G, Annoscia D, Margiotta M, Ferrara R, Varricchio P, et al. 2016. A mutualistic symbiosis between a parasitic mite and a pathogenic virus undermines honey bee immunity and health. *PNAS* 113:3203–8
62. Schmid MR, Brockmann A, Pirk CWW, Stanley DW, Tautz J. 2008. Adult honeybees (*Apis mellifera* L.) abandon hemocytic, but not phenoloxidase-based immunity. *J. Insect Physiol.* 54:439–44
63. López-Uribe MM, Appler RH, Youngsteadt E, Dunn RR, Frank SD, Tarpy DR. 2017. Higher immunocompetence is associated with higher genetic diversity in feral honey bee colonies (*Apis mellifera*). *Conserv. Genet.* 18:659–66

56. Highlights the potential of colony-level molecular biomarkers to assess the effects of landscape conservation efforts.

64. Alaux C, Allier F, Decourtye A, Odoux J-F, Tamic T, et al. 2017. A "landscape physiology" approach for assessing bee health highlights the benefits of floral landscape enrichment and semi-natural habitats. *Sci. Rep.* 7:40568
65. Alaux C, Dantec C, Parrinello H, Le Conte Y. 2011. Nutrigenomics in honey bees: digital gene expression analysis of pollen's nutritive effects on healthy and *Varroa*-parasitized bees. *BMC Genom.* 12:496
66. Di Pasquale G, Salignon M, Le Conte Y, Belzunces LP, Decourtye A, et al. 2013. Influence of pollen nutrition on honey bee health: Do pollen quality and diversity matter? *PLOS ONE* 8:e72016
67. Pokhrel V, DeLisi NA, Danka RG, Walker TW, Ottea JA, Healy KB. 2018. Effects of truck-mounted, ultra low volume mosquito adulticides on honey bees (*Apis mellifera*) in a suburban field setting. *PLOS ONE* 13:e0193535
68. Berenbaum MR, Johnson RM. 2015. Xenobiotic detoxification pathways in honey bees. *Curr. Opin. Insect Sci.* 10:51–58
69. Manjon C, Troczka BJ, Zaworra M, Beadle K, Randall E, et al. 2018. Unravelling the molecular determinants of bee sensitivity to neonicotinoid insecticides. *Curr. Biol.* 28:1137–43.e5
70. Mao W, Schuler MA, Berenbaum MR. 2011. CYP9Q-mediated detoxification of acaricides in the honey bee (*Apis mellifera*). *PNAS* 108:12657–62
71. Douglas AE. 2015. Multiorganismal insects: diversity and function of resident microorganisms. *Annu. Rev. Entomol.* 60:17–34
72. Kwong WK, Medina LA, Koch H, Sing K-W, Soh EJY, et al. 2017. Dynamic microbiome evolution in social bees. *Sci. Adv.* 3:e1600513
73. Lee FJ, Rusch DB, Stewart FJ, Mattila HR, Newton ILG. 2015. Saccharide breakdown and fermentation by the honey bee gut microbiome. *Environ. Microbiol.* 17:796–815
74. Ricigliano VA, Fitz W, Copeland DC, Mott BM, Maes P, et al. 2017. The impact of pollen consumption on honey bee (*Apis mellifera*) digestive physiology and carbohydrate metabolism. *Arch. Insect Biochem. Physiol.* 96:e21406
75. Zheng H, Powell JE, Steele MI, Dietrich C, Moran NA. 2017. Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. *PNAS* 114:4775–80
76. Rubanov A, Russell KA, Rothman JA, Nieh JC, McFrederick QS. 2019. Intensity of *Nosema ceranae* infection is associated with specific honey bee gut bacteria and weakly associated with gut microbiome structure. *Sci. Rep.* 9:3820
77. Schwarz RS, Moran NA, Evans JD. 2016. Early gut colonizers shape parasite susceptibility and microbiota composition in honey bee workers. *PNAS* 113:9345–50
78. Kwong WK, Mancenido AL, Moran NA. 2017. Immune system stimulation by the native gut microbiota of honey bees. *R. Soc. Open Sci.* 4:170003
79. Anderson KE, Ricigliano VA. 2017. Honey bee gut dysbiosis: a novel context of disease ecology. *Curr. Opin. Insect Sci.* 22:125–32
80. Mockler BK, Kwong WK, Moran NA, Koch H. 2018. Microbiome structure influences infection by the parasite *Crithidia bombi* in bumble bees. *Appl. Environ. Microbiol.* 84:e02335
81. Koch H, Schmid-Hempel P. 2012. Gut microbiota instead of host genotype drive the specificity in the interaction of a natural host-parasite system. *Ecol. Lett.* 15:1095–103
82. Powell E, Ratnayeke N, Moran NA. 2016. Strain diversity and host specificity in a specialized gut symbiont of honeybees and bumblebees. *Mol. Ecol.* 25:4461–71
83. Ellegaard KM, Engel P. 2019. Genomic diversity landscape of the honey bee gut microbiota. *Nat. Commun.* 10:446
84. Jones JC, Fruciano C, Hildebrand F, Al Toufalilia H, Balfour NJ, et al. 2018. Gut microbiota composition is associated with environmental landscape in honey bees. *Ecol. Evol.* 8:441–51
85. Rothman JA, Andrikopoulos C, Cox-Foster D, McFrederick QS. 2019. Floral and foliar source affect the bee nest microbial community. *Microb. Ecol.* 78:506–16
86. Francis RM, Nielsen SL, Kryger P. 2013. *Varroa*-virus interaction in collapsing honey bee colonies. *PLOS ONE* 8:e57540
87. Martin S. 1998. A population model for the ectoparasitic mite *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies. *Ecol. Model.* 109:267–81

88. Danka RG, Harris JW, Dodds GE. 2016. Selection of VSH-derived "Pol-line" honey bees and evaluation of their *Varroa*-resistance characteristics. *Apidologie* 47:483–90
89. de Guzman L, Rinderer TE, Frake AM. 2007. Growth of *Varroa destructor* (Acari: Varroidae) populations in Russian honey bee (Hymenoptera: Apidae) colonies. *Ann. Entomol. Soc. Am.* 100:187–95
90. Harbo JR, Harris JW. 2005. Suppressed mite reproduction explained by the behaviour of adult bees. *J. Apic. Res.* 44:21–23
91. Kirrane MJ, de Guzman LI, Holloway B, Frake AM, Rinderer TE, Whelan PM. 2015. Phenotypic and genetic analyses of the *Varroa* sensitive hygienic trait in Russian honey bee (Hymenoptera: Apidae) colonies. *PLOS ONE* 10:e0116672
92. Dietemann V, Nazzi F, Martin SJ, Anderson DL, Locke B, et al. 2013. Standard methods for *Varroa* research. *J. Apic. Res.* 52:1–54
93. Lee KV, Moon RD, Burkness EC, Hutchison WD, Spivak M. 2010. Practical sampling plans for *Varroa destructor* (Acari: Varroidae) in *Apis mellifera* (Hymenoptera: Apidae) colonies and apiaries. *J. Econ. Entomol.* 103:1039–50
94. DeGrandi-Hoffman G, Ahumada F, Danka R, Chambers M, DeJong EW, Hidalgo G. 2017. Population growth of *Varroa destructor* (Acari: Varroidae) in colonies of Russian and unselected honey bee (Hymenoptera: Apidae) stocks as related to numbers of foragers with mites. *J. Econ. Entomol.* 110:809–15
95. Delaplane KS, van der Steen J, Guzman-Novoa E. 2013. Standard methods for estimating strength parameters of *Apis mellifera* colonies. *J. Apic. Res.* 52:1–12
96. Goulson D, Hughes W, Derwent L, Stout J. 2002. Colony growth of the bumblebee, *Bombus terrestris*, in improved and conventional agricultural and suburban habitats. *Oecologia* 130:267–73

97. Study linking specific nutritional requirements to bumble bee colony health.

97. Vaudo AD, Farrell LM, Patch HM, Grozinger CM, Tooker JF. 2018. Consistent pollen nutritional intake drives bumble bee (*Bombus impatiens*) colony growth and reproduction across different habitats. *Ecol. Evol.* 8:5765–76

98. Mattila HR, Seeley TD. 2007. Genetic diversity in honey bee colonies enhances productivity and fitness. *Science* 317:362–64
99. Meikle WG, Corby-Harris V, Carroll MJ, Weiss M, Snyder LA, et al. 2019. Exposure to sublethal concentrations of methoxyfenozide disrupts honey bee colony activity and thermoregulation. *PLOS ONE* 14:e0204635
100. Meikle WG, Weiss M, Maes PW, Fitz W, Snyder LA, et al. 2017. Internal hive temperature as a means of monitoring honey bee colony health in a migratory beekeeping operation before and during winter. *Apidologie* 48:666–80
101. Huang SK, Csaki T, Doublet V, Dussaubat C, Evans JD, et al. 2014. Evaluation of cage designs and feeding regimes for honey bee (Hymenoptera: Apidae) laboratory experiments. *J. Econ. Entomol.* 107:54–62
102. Ricigliano VA, Mott BM, Floyd AS, Copeland DC, Carroll MJ, Anderson KE. 2018. Honey bees overwintering in a southern climate: longitudinal effects of nutrition and queen age on colony-level molecular physiology and performance. *Sci. Rep.* 8:10475
103. Salmela H, Stark T, Stucki D, Fuchs S, Freitak D, et al. 2016. Ancient duplications have led to functional divergence of vitellogenin-like genes potentially involved in inflammation and oxidative stress in honey bees. *Genome Biol. Evol.* 8:495–506
104. Barroso-Arévalo S, Vicente-Rubiano M, Puerta F, Molero F, Sánchez-Vizcaíno JM. 2019. Immune related genes as markers for monitoring health status of honey bee colonies. *BMC Vet. Res.* 15:72
105. Oldroyd BP, Fewell JH. 2007. Genetic diversity promotes homeostasis in insect colonies. *Trends Ecol. Evol.* 22:408–13
106. Palmer KA, Oldroyd BP. 2003. Evidence for intra-colonial genetic variance in resistance to American foulbrood of honey bees (*Apis mellifera*): further support for the parasite/pathogen hypothesis for the evolution of polyandry. *Naturwissenschaften* 90:265–68
107. Tarpy DR. 2003. Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. *Proc. R. Soc. Lond. B Biol. Sci.* 270:99–103

108. Tarpy DR, vanEngelsdorp D, Pettis JS. 2013. Genetic diversity affects colony survivorship in commercial honey bee colonies. *Naturwissenschaften* 100:723–28
109. Strassmann J. 2001. The rarity of multiple mating by females in the social Hymenoptera. *Insectes Soc.* 48:1–13
110. Cremer S, Pull CD, Fürst MA. 2018. Social immunity: emergence and evolution of colony-level disease protection. *Annu. Rev. Entomol.* 63:105–23
111. Simone-Finstrom M. 2017. Social immunity and the superorganism: behavioral defenses protecting honey bee colonies from pathogens and parasites. *Bee World* 94:21–29
112. Wilson-Rich N, Spivak M, Fefferman NH, Starks PT. 2009. Genetic, individual, and group facilitation of disease resistance in insect societies. *Annu. Rev. Entomol.* 54:405–23
113. Meunier J. 2015. Social immunity and the evolution of group living in insects. *Philos. Trans. R. Soc. B Biol. Sci.* 370:20140102
114. Momot JP, Rothenbuhler WC. 1971. Behaviour genetics of nest cleaning in honeybees. VI. Interactions of age and genotype of bees, and nectar flow. *J. Apic. Res.* 10:11–21
115. Gerdts J, Dewar RL, Simone-Finstrom M, Edwards T, Angove M. 2018. Hygienic behaviour selection via freeze-killed honey bee brood not associated with chalkbrood resistance in eastern Australia. *PLOS ONE* 13:e0203969
116. Tsvetkov N, Samson-Robert O, Sood K, Patel HS, Malena DA, et al. 2017. Chronic exposure to neonicotinoids reduces honey bee health near corn crops. *Science* 356:1395–97
117. Mondet F, Alaux C, Severac D, Rohmer M, Mercer AR, Le Conte Y. 2015. Antennae hold a key to *Varroa*-sensitive hygiene behaviour in honey bees. *Sci. Rep.* 5:10454
118. Guarna MM, Hoover SE, Huxter E, Higo H, Moon K-M, et al. 2017. Peptide biomarkers used for the selective breeding of a complex polygenic trait in honey bees. *Sci. Rep.* 7:8381
119. Harpur BA, Guarna MM, Huxter E, Higo H, Moon K-M, et al. 2019. Integrative genomics reveals the genetics and evolution of the honey bee's social immune system. *Genome Biol. Evol.* 11:937–48
120. Page RE, Robinson GE, Fondrk MK, Nasr ME. 1995. Effects of worker genotypic diversity on honey bee colony development and behavior (*Apis mellifera* L.). *Behav. Ecol. Sociobiol.* 36:387–96
121. Gilioli G, Sperandio G, Hatjina F, Simonetto A. 2019. Towards the development of an index for the holistic assessment of the health status of a honey bee colony. *Ecol. Indic.* 101:341–47
122. Richard F-J, Aubert A, Grozinger CM. 2008. Modulation of social interactions by immune stimulation in honey bee, *Apis mellifera*, workers. *BMC Biol.* 6:50
123. Wagoner KM, Spivak M, Rueppell O. 2018. Brood affects hygienic behavior in the honey bee (Hymenoptera: Apidae). *J. Econ. Entomol.* 111:2520–30
124. Simone-Finstrom M, Borba R, Wilson M, Spivak M. 2017. Propolis counteracts some threats to honey bee health. *Insects* 8:46
125. Spivak M, Goblirsch M, Simone-Finstrom M. 2019. Social-medication in bees: the line between individual and social regulation. *Curr. Opin. Insect Sci.* 33:49–55
126. Moreau SJM. 2013. "It stings a bit but it cleans well:" venoms of Hymenoptera and their antimicrobial potential. *J. Insect Physiol.* 59:186–204
127. Bucekova M, Valachova I, Kohutova L, Prochazka E, Klaudiny J, Majtan J. 2014. Honeybee glucose oxidase—its expression in honeybee workers and comparative analyses of its content and H_2O_2-mediated antibacterial activity in natural honeys. *Die Naturwiss.* 101:661–70
128. Jones B, Shipley E, Arnold KE. 2018. Social immunity in honeybees—density dependence, diet, and body mass trade-offs. *Ecol. Evol.* 8:4852–59
129. Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, et al. 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* 318:283–87
130. Evans JD, Schwarz RS. 2011. Bees brought to their knees: microbes affecting honey bee health. *Trends Microbiol.* 19:614–20
131. Schwarz RS, Huang Q, Evans JD. 2015. Hologenome theory and the honey bee pathosphere. *Curr. Opin. Insect Sci.* 10:1–7
132. McMenamin AJ, Flenniken ML. 2018. Recently identified bee viruses and their impact on bee pollinators. *Curr. Opin. Insect Sci.* 26:120–29

131. Review discusses honey bee microbial associates as an extension of the colony phenotype and host health.

133. Schwarz RS, Teixeira EW, Tauber JP, Birke JM, Martins MF, et al. 2014. Honey bee colonies act as reservoirs for two *Spiroplasma* facultative symbionts and incur complex, multiyear infection dynamics. *Microbiol. Open* 3:341–55
134. Glenny W, Cavigli I, Daughenbaugh KF, Radford R, Kegley SE, Flenniken ML. 2017. Honey bee (*Apis mellifera*) colony health and pathogen composition in migratory beekeeping operations involved in California almond pollination. *PLOS ONE* 12:e0182814
135. Pirk CWW, de Miranda JR, Kramer M, Murray TE, Nazzi F, et al. 2013. Statistical guidelines for *Apis mellifera* research. *J. Apic. Res.* 52:1–24
136. Thaduri S, Stephan JG, de Miranda JR, Locke B. 2019. Disentangling host-parasite-pathogen interactions in a *Varroa*-resistant honeybee population reveals virus tolerance as an independent, naturally adapted survival mechanism. *Sci. Rep.* 9:6221
137. Jensen AB, Pedersen BV, Eilenberg J. 2009. Differential susceptibility across honey bee colonies in larval chalkbrood resistance. *Apidologie* 40:524–34
138. McFrederick QS, Thomas JM, Neff JL, Vuong HQ, Russell KA, et al. 2017. Flowers and wild megachilid bees share microbes. *Microb. Ecol.* 73:188–200
139. Forsgren E, Olofsson TC, Vásquez A, Fries I. 2010. Novel lactic acid bacteria inhibiting *Paenibacillus larvae* in honey bee larvae. *Apidologie* 41:99–108
140. Vásquez A, Forsgren E, Fries I, Paxton RJ, Flaberg E, et al. 2012. Symbionts as major modulators of insect health: lactic acid bacteria and honeybees. *PLOS ONE* 7:e33188
141. Lebuhn G, Droege S, Connor EF, Gemmill-Herren B, Potts SG, et al. 2013. Detecting insect pollinator declines on regional and global scales. *Conserv. Biol.* 27:113–20
142. Batáry P, Báldi A, Kleijn D, Tscharntke T. 2011. Landscape-moderated biodiversity effects of agri-environmental management: a meta-analysis. *Proc. R. Soc. B Biol. Sci.* 278:1894–902
143. Whitehorn PR, Tinsley MC, Brown MJF, Darvill B, Goulson D. 2011. Genetic diversity, parasite prevalence and immunity in wild bumblebees. *Proc. R. Soc. B Biol. Sci.* 278:1195–202
144. Jaffé R, Pope N, Acosta AL, Alves DA, Arias MC, et al. 2016. Beekeeping practices and geographic distance, not land use, drive gene flow across tropical bees. *Mol. Ecol.* 25:5345–58
145. López-Uribe MM, Jha S, Soro A. 2019. A trait-based approach to predict population genetic structure in bees. *Mol. Ecol.* 28:1919–29
146. Lozier J. 2014. Revisiting comparisons of genetic diversity in stable and declining species: assessing genome-wide polymorphism in North American bumble bees using RAD sequencing. *Mol. Ecol.* 23:788–801
147. López-Uribe MM, Cane JH, Minckley RL, Danforth BN. 2016. Crop domestication facilitated rapid geographical expansion of a specialist pollinator, the squash bee *Peponapis pruinosa*. *Proc. R. Soc. B Biol. Sci*. 283:20160443
148. Ogilvie JE, Forrest JRK. 2017. Interactions between bee foraging and floral resource phenology shape bee populations and communities. *Curr. Opin. Insect Sci.* 21:75–82
149. Requier F, Odoux J-F, Tamic T, Moreau N, Henry M, et al. 2015. Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. *Ecol. Appl.* 25:881–90
150. Vaudo AD, Tooker JF, Grozinger CM, Patch HM. 2015. Bee nutrition and floral resource restoration. *Curr. Opin. Insect Sci.* 10:133–41
151. Whitehorn PR, O'Connor S, Wackers FL, Goulson D. 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* 336:351–52
152. Gill RJ, Ramos-Rodriguez O, Raine NE. 2012. Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature* 491:105–8
153. Meeus I, Pisman M, Smagghe G, Piot N. 2018. Interaction effects of different drivers of wild bee decline and their influence on host-pathogen dynamics. *Curr. Opin. Insect Sci.* 26:136–41
154. Tosi S, Nieh JC, Sgolastra F, Cabbri R, Medrzycki P. 2017. Neonicotinoid pesticides and nutritional stress synergistically reduce survival in honey bees. *Proc. R. Soc. B Biol. Sci.* 284:20171711
155. Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, et al. 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *PLOS ONE* 5:e9754

154. Study provides mechanistic evidence that two major stressors, field-realistic nutritional deficiency and pesticide exposure, can synergistically interact.

156. Reeves AM, O'Neal ST, Fell RD, Brewster CC, Anderson TD. 2018. In-hive acaricides alter biochemical and morphological indicators of honey bee nutrition, immunity, and development. *J. Insect Sci.* 18:8
157. Mussen EC, Lopez JE, Peng CYS. 2004. Effects of selected fungicides on growth and development of larval honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). *Environ. Entomol.* 33:1151–54
158. Raymann K, Bobay L-M, Moran NA. 2018. Antibiotics reduce genetic diversity of core species in the honeybee gut microbiome. *Mol. Ecol.* 27:2057–66
159. McArt SH, Urbanowicz C, McCoshum S, Irwin RE, Adler LS. 2017. Landscape predictors of pathogen prevalence and range contractions in US bumblebees. *Proc. R. Soc. B Biol. Sci.* 284:20172181
160. Dolezal AG, Toth AL. 2018. Feedbacks between nutrition and disease in honey bee health. *Curr. Opin. Insect Sci.* 26:114–19
161. O'Neal ST, Anderson TD, Wu-Smart JY. 2018. Interactions between pesticides and pathogen susceptibility in honey bees. *Curr. Opin. Insect Sci.* 26:57–62
162. Gibbs EPJ. 2014. The evolution of One Health: a decade of progress and challenges for the future. *Vet. Rec.* 174:85–91
163. Eilers EJ, Kremen C, Smith Greenleaf S, Garber AK, Klein A-M. 2011. Contribution of pollinator-mediated crops to nutrients in the human food supply. *PLOS ONE* 6:e21363
164. Smith MR, Singh GM, Mozaffarian D, Myers SS. 2015. Effects of decreases of animal pollinators on human nutrition and global health: a modelling analysis. *Lancet* 386:1964–72
165. Power AG. 2010. Ecosystem services and agriculture: tradeoffs and synergies. *Philos. Trans. R. Soc. B Biol. Sci.* 365:2959–71
166. Klein A-M, Boreux V, Fornoff F, Mupepele A-C, Pufal G. 2018. Relevance of wild and managed bees for human well-being. *Curr. Opin. Insect Sci.* 26:82–88
167. Cane JH, Sipes S. 2006. Characterizing floral specialization by bees: analytical methods and a revised lexicon for oligolecty. In *Plant-Pollinator Interactions: From Specialization to Generalization*, ed. NM Waser, J Ollerton, pp. 99–122. Chicago: Univ. Chicago Press
168. Cane JH. 1991. Soils of ground-nesting bees (Hymenoptera: Apoidea): texture, moisture, cell depth and climate. *J. Kans. Entomol. Soc.* 64:406–13
169. Harbo JR, Harris JW. 2009. Responses to *Varroa* by honey bees with different levels of Varroa Sensitive Hygiene. *J. Apic. Res.* 48:156–61
170. de Miranda JR, Bailey L, Ball BV, Blanchard P, Budge GE, et al. 2013. Standard methods for virus research in *Apis mellifera*. *J. Apic. Res.* 52:1–56
171. Evans JD, Schwarz RS, Chen YP, Budge G, Cornman RS, et al. 2013. Standard methods for molecular research in *Apis mellifera*. *J. Apic. Res.* 52:1–54
172. Traver BE, Fell RD. 2011. Prevalence and infection intensity of *Nosema* in honey bee (*Apis mellifera* L.) colonies in Virginia. *J. Invertebr. Pathol.* 107:43–49
173. Cavigli I, Daughenbaugh KF, Martin M, Lerch M, Banner K, et al. 2016. Pathogen prevalence and abundance in honey bee colonies involved in almond pollination. *Apidologie* 47:251–66
174. Schurr F, Cougoule N, Rivière MP, Ribière-Chabert M, Achour H, et al. 2017. Trueness and precision of the real-time RT-PCR method for quantifying the chronic bee paralysis virus genome in bee homogenates evaluated by a comparative inter-laboratory study. *J. Virol. Methods* 248:217–25
175. Fries I, Chauzat MP, Chen YP, Doublet V, Genersch E, et al. 2013. Standard methods for *Nosema* research. *J. Apic. Res.* 52:1–28
176. Sammataro D, de Guzman L, George S, Ochoa R, Otis G. 2013. Standard methods for tracheal mite research. *J. Apic. Res.* 52:1–20
177. Meikle WG, Holst N. 2015. Application of continuous monitoring of honeybee colonies. *Apidologie* 46:10–22
178. Sagili RR, Pankiw T, Keyan ZS. 2005. Effects of soybean trypsin inhibitor on hypopharyngeal gland protein content, total midgut protease activity and survival of the honey bee (*Apis mellifera* L.). *J. Insect Physiol.* 51:953–57
179. Wilson-Rich N, Dres ST, Starks PT. 2008. The ontogeny of immunity: development of innate immune strength in the honey bee (*Apis mellifera*). *J. Insect Physiol.* 54:1392–99

166. Review of the evidence that bees contribute not only to food security via pollination but also to a wide range of benefits for human well-being, including medical resources and human spiritual practices.

180. López-Uribe MM, Sconiers WB, Frank SD, Dunn RR, Tarpy DR. 2016. Reduced cellular immune response in social insect lineages. *Biol. Lett.* 12:20150984
181. Evans JD, Pettis JS. 2005. Colony-level impacts of immune responsiveness in honey bees, *Apis mellifera*. *Evolution* 59:2270–74
182. Praet J, Parmentier A, Schmid-Hempel R, Meeus I, Smagghe G, Vandamme P. 2018. Large-scale cultivation of the bumblebee gut microbiota reveals an underestimated bacterial species diversity capable of pathogen inhibition. *Environ. Microbiol.* 20:214–27
183. Martinson VG, Moy J, Moran NA. 2012. Establishment of characteristic gut bacteria during development of the honeybee worker. *Appl. Environ. Microbiol.* 78:2830–40
184. McFrederick QS, Rehan SM. 2019. Wild bee pollen usage and microbial communities co-vary across landscapes. *Microbial Ecol.* 77:513–22
185. Guarna MM, Melathopoulos AP, Huxter E, Iovinella I, Parker R, et al. 2015. A search for protein biomarkers links olfactory signal transduction to social immunity. *BMC Genomics* 16:63