Ecological and evolutionary approaches to managing honeybee disease

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Honeybee declines are a serious threat to global agricultural security and productivity. Although multiple factors contribute to these declines, parasites are a key driver. Disease problems in honeybees have intensified in recent years, despite increasing attention to addressing them. Here we argue that we must focus on the principles of disease ecology and evolution to understand disease dynamics, assess the severity of disease threats, and control these threats via honeybee management. We cover the ecological context of honeybee disease, including both host and parasite factors driving current transmission dynamics, and then discuss evolutionary dynamics including how beekeeping management practices may drive selection for more virulent parasites. We then outline how ecological and evolutionary principles can guide disease mitigation in honeybees, including several practical management suggestions for addressing short- and long-term disease dynamics and consequences.

Pollinator declines are a major societal challenge. In particular, managed populations of the western honeybee are facing unprecedented declines[1–4]. In the US, the number of honeybee colonies has been reduced by half since 1940 (Fig. 1) in tandem with a large increase in the acreage of honeybee dependent crops[5]. Over the past several years, there have been annual losses of between 30 and 40% of all managed US honeybee colonies[6], corresponding to over 1 million colonies lost per year[5,7], alongside similar losses in Europe[8] (Fig. 1). These colony losses are detrimental to pollination services[9,10], threatening human health[11] and hundreds of billions of dollars in agricultural value[12]. Honeybees are the most important managed pollinators worldwide[13,14], contributing to the production of 39 of the 57 leading crops used for human consumption[15]. Many of these crops comprise the fruits, nuts, seeds and vegetables that provide the bulk of micronutrients to the human diet[11,16,17].

Although many factors contribute to honeybee declines, such as pesticides and land-use change[18,19], we focus here on parasites (Box 1). A wide range of parasites cause significant threats to honeybees, including viruses, bacteria, microsporidia, and arthropods[7,20–24] (Fig. 2 and Table 1). While colony losses have often been ascribed to colony collapse disorder—a syndrome associated with a loss of adult workers, a lack of dead or diseased bees in or near the colony, and the delayed invasion of nest scavengers—this disorder is hard to define, and its causes remain unclear. What is clear, however, is that many colony losses are due to parasites, whether alone, together, or in combination with other factors such as pesticides. In particular, the ectoparasitic mite *Varroa destructor* and the viruses it vectors are considered the primary cause of honeybee colony losses worldwide[1,25–31].

Why have honeybee disease management efforts had so little success? Early efforts (mid-2000s) took an 'outbreak mentality,' trying to identify a single disorder driving declines[32]. More recent efforts have examined interactions between parasites[24,26,33], or between parasites and other stressors[18,34,35]. Here, we argue that there is a need to better incorporate the principles of ecology and evolution to reveal fundamental causes of declines, predict factors that increase the severity of threats, and underpin knowledge-based solutions. We start by outlining ecological factors regulating honeybee disease, including host density and heterogeneity; host

behaviour including ecological and social immunity; and community ecology. We then move to evolutionary processes, arguing that current beekeeping practices not only increase the prevalence and severity of disease outbreaks, but also thus promote natural selection for more virulent (harmful) parasites. We outline how an ecological and evolutionary framework can provide specific recommendations to mitigate disease threats and point out emerging research directions and challenges.

Our focus in this Review is on disease. Still, we underscore that to completely reverse honeybee declines other stressors such as pesticides, lack of forage, and land-use practices must be addressed[18,34–37].

Ecological considerations
Host density is a key consideration for understanding the ecological dynamics of infectious disease.

Host density and infectious disease spread. The importance of population density for disease ecology is shown clearly in theoretical epidemiological models, which have been crucial to understanding infectious disease dynamics and control measures in humans, wildlife and agriculture[38–42]. These include SI and SIR models, based on the compartmentalization of each individual in the host population into susceptible (S), infected (I), or recovered and immune (R) classes (Box 2). Individual hosts move between compartments based on rates of transmission (from S to I) or recovery (from I to R). Despite the abstraction and simplicity of these models, they have been successful at reproducing disease dynamics in systems ranging from rabies in wild foxes[43] to Ebola in humans[41], to foot and mouth disease in livestock[42].

Host population density is a crucial determinant of transmission, parasite spread and epidemic size. Parasites are not only more likely to invade denser populations, but are also less likely to go extinct following initial epidemics[44,45]. The importance of host population density was first recognized by Kermack and McKendrick[38] and verified by studies on measles in which larger cities showed greater and more frequent measles outbreaks and greater rates of endemism than smaller cities[46,47]. This increased risk is driven by higher densities of susceptible individuals, higher colonization rates by diseased individuals[48] and higher contact rates[49].

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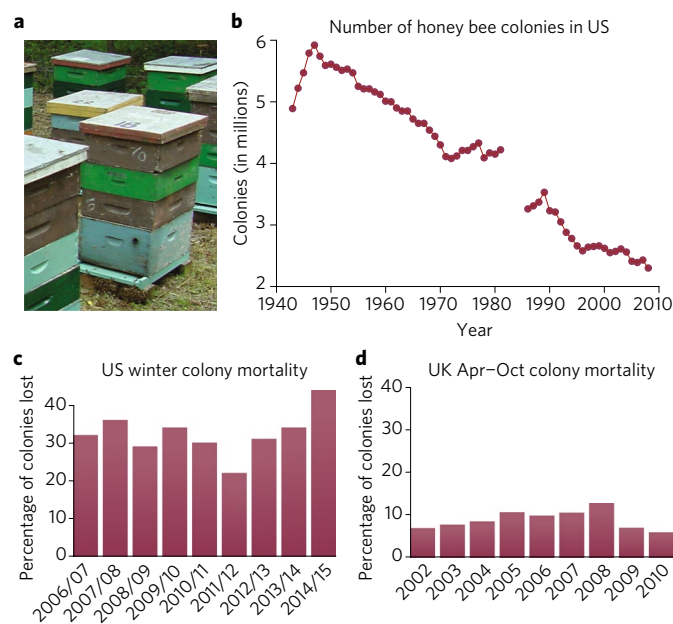
Fig. 1 | Honeybee colony losses. a, A typical managed honeybee colony. b, Colony numbers (in millions) in the United States between 1943 and 2016[5]. c, United States winter colony losses between 2006 and 2015[6,211]. d, Breeding season colony losses in England and Wales between 2002 and 2010[7]. Data in c,d were obtained from graphs in their respective source papers[5,7] using WebPlotDigitizer (http://arohatgi.info/WebPlotDigitizer/app/). Photo credit: K.S.D.

High host density is thus a key factor that has contributed to increased parasite prevalence and incidence in honeybees. In modern beekeeping, this problem expresses at two levels—worker populations within colonies, and colony densities (apiaries) at a landscape scale. Larger colony populations are associated with more honey hoarding[50], which has driven beekeeping interventions such as swarm prevention (discouraging colony reproductive fission), stimulatory feeding, and chemo-centric disease and parasite control to achieve unnaturally large forager populations and honey crops[51]. As a result, while peak-season worker populations in healthy natural colonies in hollow trees are about 18,000[52], reported peak-season populations in healthy managed colonies range from

Box 1 | Parasites, pests and pathogens

Different authors and scientific fields use different definitions of parasites, pests and pathogens. In the parasitological literature, parasites generally refer to protozoans and worms. Although epidemiologists may refer to protozoans, bacteria and viruses as pathogens, ecologists will often include bacteria and viruses in their broad definition of parasites. In the animal literature, the term 'pest' is often reserved for organisms that do not directly extract resources from hosts, but instead parasitize their habitats, such as nests and food sources.

In this Review we use the term 'parasite' to broadly refer to organisms that reduce the fitness of honeybees, either at the individual or colony level. Thus, parasites include any organism ranging from the deformed wing virus, which infects the haemolymph of bees and causes deformed wings, to Varroa destructor mites, which suck the blood of bee larvae, and small hive beetles, which parasitize the honey and pollen stores of honeybee colonies.

20,000–52,000[50,53,54]. In addition, the average volume of natural tree hollows occupied by bees ranges from 15–80 litres[55], while with US beekeeping equipment the standard 'Langstroth hive body'—the basic module of a hive—contains a volume of 43 l. Beekeepers adjust module number as needed to accommodate seasonal fluxes in incoming honey, resulting in nest volumes ranging from 43–172 l. Although higher population sizes do not necessarily translate to higher within-colony densities, it is still likely that these practices support unnaturally high densities, and some specialty practices—such as the production of comb honey—call for radically dense worker populations[56]. The fact that simulated 'natural' colonies (approx. 10,000 bees kept year-round in single 43 l hive bodies and left free to swarm) showed lower levels of Varroa mites and brood diseases compared with conventionally managed colonies (approx. 30,000 bees managed for swarm control)[54] suggests that worker density within colony deserves more attention in the context of ecologically informed health management.

Moving from single colonies to apiaries (collections of multiple colonies in one location), beekeepers routinely maintain colonies at much greater densities in apiaries than occur in nature (Fig. 3a,b). While feral bee colonies occur at a density of around one per square kilometre[57], industrial beekeeping operations may maintain thousands of colonies in a similar space. One study on Varroa compared an apiary of 12 bee colonies with 12 colonies scattered throughout the landscape, and found lower two-year colony survival in the apiary than in scattered colonies, as well as greater rates of drone drifting (that is, male bees entering non-home colonies) between colonies, consistent with greater Varroa transmission[58]. Between-colony (horizontal) mite transmission has been independently supported[59–61], but the evidence is not always equivocal[62]. In order to better understand the importance of colony density in disease processes, more studies are necessary. For example, we know relatively little of the importance of dormancy in parasites (such as the bacterial causative agents of foulbrood) to transmission in heavily managed apiaries.

Despite the theoretical benefits of low host density, economic and cultural pressures have moved the beekeeping industry toward larger, denser apiaries. It is difficult to imagine significant departures from this paradigm unless cost–benefit analyses show that benefits of improved bee health significantly exceed costs from increased swarming and more scattered apiaries.

Demography and disease spread. Theoretical studies have shown that population demography crucially affects disease epidemics. Rapid demographic turnover, where new susceptible individuals are added to a population at high rates, leads to a greater probability of maintaining disease and sustaining epidemics over longer periods of time[44,45]. Modern day beekeepers do just that, by replacing succumbed colonies with new ones, thus artificially replenishing the population with susceptible hosts. This practice is facilitated by the use of 'package' bees (one queen with several pounds of workers) and 'nucleus' colonies (small colonies with a queen, 1–2 pounds of bees, and 4–5 combs of brood), hundreds of thousands of which are shipped annually to beekeepers for restocking dead colonies. This results in very different demographic dynamics relative to natural disease systems, where susceptibles are used up as an epidemic burns through a population.

Population heterogeneity. Theoretical and empirical studies have shown that genetically homogeneous populations of susceptible hosts are often prone to parasite invasion and rapid parasite spread. In contrast, in genetically variable populations, specialist parasites are less likely to spread, potentially resulting in less-severe disease in fewer individuals[63]. This is true at the species level as well, that is, more diverse communities often reduce the infection of susceptible host species[64,65]. Similarly, intercropping and crop rotation in agriculture have been successful strategies for reducing the impacts of parasites[66,67].
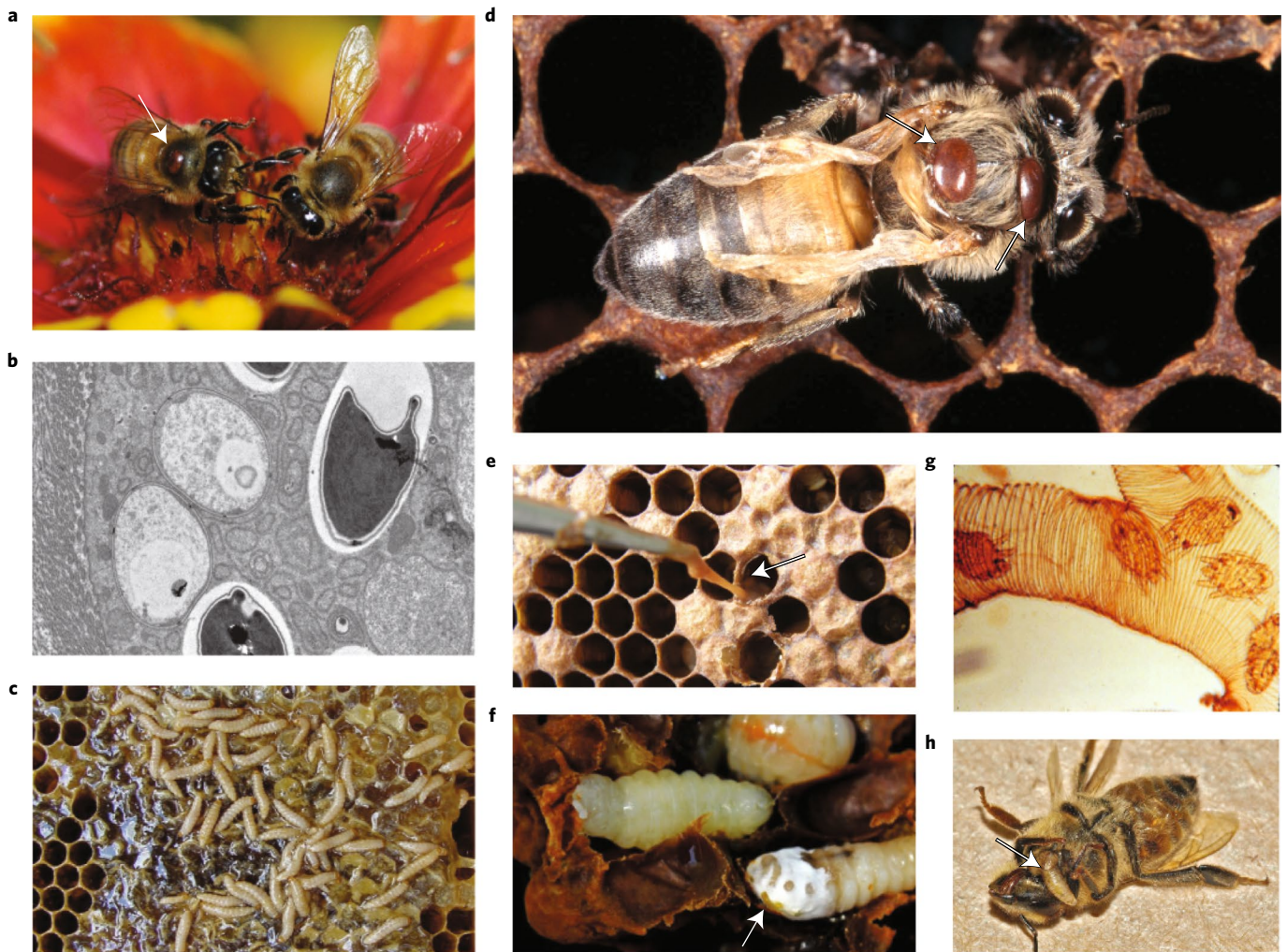
Fig. 2 | Parasites threatening honeybees. a, *Varroa destructor* mite (indicated with arrow) attached to a foraging worker bee. **b**, Spores of the microsporidian *Nosema ceranae* in a honeybee ventricular cell. **c**, Small hive beetle (*Aethina tumida*) larvae infesting a honeybee colony frame. **d**, A honeybee with deformed wings, caused by infection with deformed wing virus; the arrows point at *Varroa* mites, which vector and amplify the virus. **e**, A disintegrating honeybee pupa as a result of infection with the American foulbrood bacterium *Paenibacillus larvae*. **f**, Honeybee pupa infected with the fungus *Ascosphaera apis*, which causes chalkbrood disease. **g**, Tracheal mites (*Acarapis woodi*) infesting honeybee tracheae. **h**, A parasitoid fly (*Apocephalus borealis*) larva (arrow) bursting out of an infected honeybee. Photo credits: Jennifer Berry (**a**); Mariano Higes (**b**); Jamie Ellis (**c**); Paul Kruse at KnackbockBlog (**d**); Western Australian Agriculture Authority (Department of Agriculture and Food, WA (**e**); Ron Snyder at the Bee Informed Partnership (**f**); USDA (**g**); John Hafernik (**h**).

Host heterogeneity is particularly important in honeybees, whose natural history is defined by eusociality. Even wild honeybee colonies naturally have high within-colony individual densities, in turn creating naturally high disease pressure[68]. In the context of host heterogeneity, this seems to be exacerbated by genotypic homogeneity: all of the individuals within a colony are siblings, borne from the same mother (the queen). But honeybees have an unusual mating system: queens are highly polyandrous, mating with an average of 12 males[69], allowing for relatively high within-colony genotypic diversity and a measure of resistance heterogeneity. Colonies headed by queens fertilized by greater numbers of drones can experience lower infestation rates with the American foulbrood bacterium *Paenibacillus larvae*, the chalkbrood fungus *Ascosphaera apis*, and the *Varroa* mite[70–72]. This is probably due to the presence of genetically resistant individuals within a colony. Thus, increasing genotypic variability within honeybee colonies could provide an effective way to control parasites.

Ecological and social immunity. Behaviour of individual animals is another ecological factor that can have dramatic effects on disease outcomes. Many animals modulate their ability to prevent, resist, or tolerate infections with behavioural changes including changes in diet. Many immune responses are energetically expensive, and there is an extensive literature demonstrating trade-offs between immune and other fitness-related functions under starvation or caloric restriction[73–76]. In beekeeping, ensuring sufficient food is a long-standing goal, and starvation is a common cause of colony loss[77].

The theory of ecological immunology, however, gives us insights into the role of diet composition, as well as other behavioural elements, in fighting disease. Recent work shows that commercial protein supplements are associated with lower colony overwintering survival and higher parasite loads relative to pollen from a single plant source[78]. Dietary diversity also matters: more-diverse pollen diets are positively related to haemocyte concentration and phenoloxidase activity in honeybees, two aspects of insect immunity[79].

A cornerstone of behavioural defence is 'self-medication', or the use of other species (typically plants and fungi and their associated microbiota) internally or externally, to protect against parasites[80]. For example, sheep and goats can reduce gastrointestinal nematode infection by selecting food with medicinal compounds, suggesting

Table 1 | Parasites of honeybees

Parasite	Parasite type	Associated disease or disorder	Evidence for associations with disease/disorder and for the establishment of parasite as etiological agent
Varroa destructor	Mite	Varroosis	Artificial inoculation of colonies led to colony losses[104]. Varroosis stems from the synergistic effects of mite growth, honeybee immune suppression and virus vectoring[26], but statistical analysis showed that growth of the mite alone contributes to colony collapse[25].
Acarapis woodi	Mite	Isle of Wight disease	High levels of mite infestation were associated with high winter colony mortality[213]. Artificial infection confirmed mortality of individual bees[22]. Modeling suggested that mite infestation alone is enough to cause colony mortality[214], but a field study did not find negative colony effects following experimental inoculation[104].
Nosema ceranae	Microsporidian	Nosemosis type C, colony collapse disorder	Presence of *N. ceranae* was associated with smaller colony size[215] or colony collapse disorder in some studies[33] but not others[27]. Experimental infection resulted in mortality of individual honeybees[181] as well as colony collapse[216].
Nosema apis	Microsporidian	Nosemosis type A, colony collapse disorder	*N. apis* was more prevalent in collapsing than non-collapsing colonies[105]. Experimental infection resulted in parasite reproduction in bees, but no obvious mortality was observed[217].
Ascosphaera apis	Fungus	Chalkbrood	Experimental infection led to increased disease incidence within colonies, reduced bee larva survival and reduced colony size[71,218].
Paenibacillus larvae	Bacterium	American foulbrood	Experimental inoculation led to bacterial infections and reduced colony size[70].
Israeli acute paralysis virus (IAPV)	Virus	Colony collapse disorder, paralysis	IAPV was associated with colony collapse disorder in some studies[32], but not others[27,105]. Experimental injection resulted in paralysis and death of individual bees[219].
Deformed wing virus (DWV)	Virus	Varroosis, colony collapse disorder	DWV presence was associated with smaller colony size[215], and the virus was more prevalent in collapsing colonies[105,220]. Experimental feeding of honeybee larvae by *Varroa* mites resulted in high replication of the virus and experimental infection with DWV resulted in high bee larval mortality[26].
Kashmir bee virus (KBV)	Virus	Colony collapse disorder	KBV was more prevalent and occurred at higher titers in collapsed colonies[27,105]. A study in which KBV was quantified with the same assay as ABPV and IAPV also showed higher prevalence in collapsing colonies[220].
Black queen cell virus (BQCV)	Virus	Colony collapse disorder	BQCV presence was associated with smaller colony size[215], and was more abundant in collapsing colonies[105]. Experimental infection with BQCV did not result in increased worker or drone mortality[221].
Acute bee paralysis virus (ABPV)	Virus	Colony collapse disorder	ABPV was more abundant in collapsing colonies[105]. Experimental injection resulted in paralysis and death of individual bees[219].
Chronic bee paralysis virus (CBPV)	Virus	Chronic paralysis syndrome	Experimental infection resulted in viral replication and bee mortality[100].
Sacbrood virus (SBV)	Virus	Sacbrood	Experimental infection caused sacbrood in honeybee larvae[222].
Apis iridescent virus (AIV)	Virus	Colony collapse disorder	AIV was associated with colony collapse disorder[33], but this association has been suggested to occur due to methodological error[223]. Experimental infection with a closely related virus resulted in bee mortality[33].
Apocephalus borealis	Phorid fly	Hive abandonment	Parasitized bees leave their colonies at night and die shortly thereafter[20], similar to one of the symptoms of colony collapse disorder. Phorid fly larvae often carry deformed wing virus and *Nosema ceranae*, suggesting a potential role of this fly in transmission of these parasites.
Aethina tumida	Nitidulid beetle	Frame collapse and swarming	Small hive beetle larvae feed on honey and pollen. High reproduction can lead to frame collapse, after which the bee colony often leaves the nest by swarming[224].

Following Koch's postulates[88,212], many studies have established particular pathogens and parasites as the etiological agent of disease by demonstrating that infection reproduces the symptoms associated with the disease. We define parasites to include pathogens and parasites that exert their negative effects at either the level of the individual bee or colony.

that varied diets—as occur in nature but more rarely in managed systems—could simultaneously reduce disease and reduce selection for drug resistance (due to reduced drug use)[81]. This ability is not limited to vertebrates, and many insects utilize self-medication[82]. Honeybees infected with *Nosema ceranae* preferred sunflower honey over honeydew honey in dual-choice tests; sunflower honey had greater antimicrobial activity and reduced the numbers of *N. ceranae* spores in the bee gut[83].

In honeybees, immune modulation and self-medication can relate not only to individual-level defences, but also to group- or

Box 2 | Infectious disease epidemiology

Classic dynamical models of pathogen infections track host classes, such as susceptible and infected individuals and therefore ignore explicit within-host dynamics. Such compartment models may have many different host classes tracking recovered and immune or exposed individuals. A simple susceptible and infected SI model is shown as follows[117,225]:
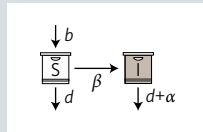
This model assumes that hosts are born at rate b, and die naturally at rate d. Infected hosts die at an additional death rate α, often referred to as virulence. When susceptible and infected hosts make contact, susceptible hosts (S) turn into infected hosts (I) at rate β.

For a parasite that is driven by density-dependent transmission, the dynamics of this model can be captured by two coupled differential equations:

$$\frac{dS}{dt} = b(S+I) - dS - \beta SI$$

$$\frac{dI}{dt} = \beta SI - (\alpha + d)I$$

One of the most useful quantities of compartment models is the basic reproductive ratio, R_0, which can be thought of as the number of new infected cases generated from one infection in a completely susceptible population. The equation for R_0 can be derived by setting $dI/dt > 0$, to determine the condition that a parasite will spread in the host population.

$$\beta SI - (\alpha + d)I > 0 \iff$$

$$\frac{\beta S}{(\alpha + d)} > 1 \iff$$

$$R_0 = \frac{\beta S}{(\alpha + d)}$$

As is clear from this equation, when R_0 exceeds 1, a parasite will be able to spread in a host population; when it is below 1, it will tend to go extinct. Due to stochastic processes, parasites with R_0 values below 1 may still spread, but control efforts are focused on bringing R_0 below 1 to curb an epidemic, for example by vaccinating or culling hosts or reducing contact between host individuals. Moreover, as this equation makes clear, R_0 increases with greater transmission rate and greater host density. It is therefore predicted that parasites are more likely to invade, and less likely to go extinct, in host populations of greater density[44].

With respect to honeybees, SI-type models may be used to study parasite spread between colonies, taking honeybee colonies as the susceptible and infected 'individuals'. Similar approaches have been used to model the spread of parasites in other agricultural practices, for example by taking farms or fields as the individual 'hosts' to model foot-and-mouth disease in livestock and rhizomania disease in sugar beet, respectively[184,185]. Considering colonies as the individual hosts allows for the study of arthropods, such as *Varroa* mites, which replicate within colonies, cause virulence at the colony level and transmit between colonies. Models can also be designed to study disease dynamics within colonies, or nested models can be created to link disease dynamics at the within- and between-colony levels[205,226].
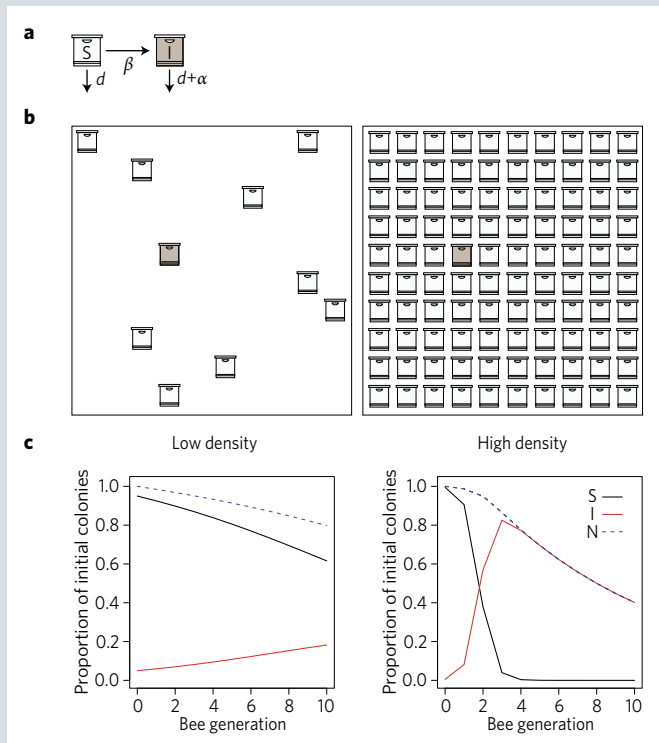
The importance of hive density on infectious disease spread. **a**, A simple disease dynamics model can be applied to honeybee parasites, in which colonies are either susceptible (S) or infected (I) with the parasite. Colonies die at a background rate **d** and become infected at the rate βSI, in which β is the transmission parameter. Infected colonies experience an additional mortality rate, α, due to parasite infection. In this model, no new colonies are added, thus representing a situation in which disease dynamics are studied in apiaries with a fixed starting density of colonies. **b**, Graphical representation of colonies maintained at low density (left) and high density (right). **c**, Disease dynamics over 20 bee generations based on the model shown in panel **a**, and with low density (20 colonies per apiary) or high density (200 colonies per apiary). Densities of susceptible (S), infected (I) and total (N) colonies are shown. At higher densities, parasites spread much more rapidly and cause greater proportional colony losses. (Parameter values used: I(0) = 1, d = 0.01, β = 0.15, α = 0.1.)

colony-level defences, that is, 'social immunity'. Social immune mechanisms in both ants and honeybees include lining nest walls with plant-produced resins (called propolis by beekeepers), which reduces infection with fungal parasites and microorganisms[84,85]. Other mechanisms include removal of dead and diseased workers

from colonies and 'social fever' or behaviourally induced increases in colony temperature to combat parasites[86]. We know little about the potentially wide range of social immune mechanisms in honeybees and how to support those mechanisms via management, but some studies have begun to address this topic. For example,
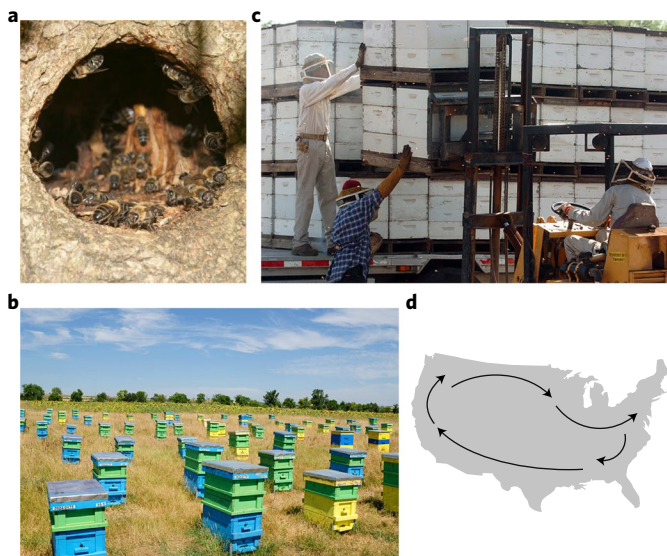
Fig. 3 | Beekeeping results in high bee densities and movement. a, Wild and feral honeybees, that is, those that have escaped management and are living outside of the realm of direct human influence, live in single colonies that are typically hyperdispersed in the landscape[156]. **b**, At the other extreme, intensively managed bees are kept in hyperconcentrated bee yards with hundreds or even thousands of colonies. **c**,**d**, Most of these operations are migratory, moving their colonies several thousand miles around states, countries and continents to provide pollination services for seasonal crops[12]. It is estimated that between one-half and two-thirds of all managed colonies in the US are moved to the almond orchards of California's Central Valley in late February and early March. Thus, transmission potential among these bees can be thought of as nearly global, since long-range movement is combined with high potential for contacting other bee colonies. Photo credits: Vitaliy Parts/Alamy Stock Photo (**a**); Dariya Angelova/Alamy Stock Photo (**b**); ZUMA Press, Inc./Alamy Stock Photo (**c**).

beekeepers can maintain, rather than remove, propolis in colonies[84,85]; and higher dietary pollen diversity increases the activity of glucose oxidase, an enzyme used to produce hydrogen peroxide for sterilizing food stores[79].

As with many other insects, studies have shown that honeybees can pass on immunity to their offspring, a process called transgenerational immune priming. For example, the larvae of queens that had been challenged with *Paenibacillus larvae*, the bacterium causing American foulbrood, experienced lower mortality than larvae of queens that had not been challenged[87]. Such transgenerational immune priming could potentially be used to immunize bee colonies[87].

Disease community ecology. Host–parasite interactions are often strongly influenced by the larger biological community[88,89]. Community ecology theory emphasizes two key interspecific interactions that are important for disease outcomes: (1) co-infections with other parasites; and (2) beneficial symbioses.

Co-infections with multiple parasites have important implications for parasite spread, disease severity and parasite evolution[90–94]. Interactions between parasites or effects on virulence can express either positively or negatively, through immune suppression or within-host competition[95–98]. In honeybee pupae with mixed infections of Kashmir bee virus, sacbrood virus and black queen cell virus, inactivation of the first two viruses resulted in activation of the third, suggesting antagonistic interactions between viruses[99]. By contrast, co-inoculation of experimental bees with *Nosema ceranae* increased the infectivity of acute bee paralysis virus[100], possibly

due to honeybee immune suppression by *N. ceranae*[101]. Beyond the individual level, co-infections are common at the colony level in honeybees, including different species of viruses, microsporidians, bacteria and mites[7,32,102]. Increasing numbers of the nest-invading small hive beetle *Aethina tumida* are associated with decreasing levels of *Varroa*[103]. In contrast, co-infection with *Varroa* and tracheal mites (*Acarapis woodi*) results in faster colony collapse than infestation with *Varroa* alone[104]. Colony collapse disorder is more often associated with multiple parasites than with single parasites[27,105], and a study that inoculated bees with a mixture of four viruses showed elevated bee mortality[24].

Varroa has been linked to honeybee declines around the world, and a major reason for this is the synergistic negative effects of *Varroa* and the viruses it vectors. Honeybee colony losses increased following the introduction of *Varroa* into the United States in the 1970s and 80s[106]. Studies in Hawaii and New Zealand have demonstrated a link between *Varroa* invasion, increasing titres of viruses including deformed wing virus and Kashmir bee virus, and subsequent colony collapses[3,107]. Indeed, phylogenetic analyses of deformed wing virus indicate that the spread of *Varroa* around the world, caused by international bee trade, is responsible for the ongoing global epidemic of this virus[108]. *Varroa* is not only a vector of this virus, but enhances its infection and growth in bees by suppressing bee immunity[109]. Statistical analyses indicate that colony collapses in colonies infected with *Varroa* and deformed wing virus are not entirely due to viral infections, but also due to the damage done by the growth of *Varroa* itself [25], thus further demonstrating the synergistic negative effects of co-infection with these parasites[26].

Beyond co-infection, the presence of other organisms, such as beneficial symbionts, can provide protection against parasites[110,111]. In humans, intact gut microbial communities (together comprising the gut 'microbiome') provide protection against parasite infection, both through immunomodulation and direct interference between commensal gut bacteria and invading parasites[112]. Similarly, the honeybee gut harbours bacteria that are antagonistic to parasites[113] such as *Ascosphaera apis*[114]. Such antagonistic interactions could provide tools for disease intervention. For example, inoculation of bee colonies with the bacterium *Parasaccharibacter apium* resulted in lower levels of *Nosema ceranae* infestation[115].

Evolutionary considerations
Although ecological factors are crucial in determining the size, duration and severity of disease outbreaks, it is equally important to consider how evolution can shape disease outcomes. We focus here on the evolution of virulence, as it is probably a key driver of disease pressures in honeybees. We begin by discussing basic theory, and then discuss how host spatial structure and population heterogeneity affect virulence evolution; how imperfect treatments can affect virulence evolution; and the importance of cross-species transmission for virulence evolution.

Virulence-transmission trade-offs. Conventional wisdom on disease evolution held that given enough time, parasites would become benign to their host[116]. However, theoretical advancements over the past four decades have recognized that virulence is instead closely linked to parasite transmission[39,117–119]. Between-host transmission increases with increasing host exploitation rate but decreases with increasing clearance by the host as well as host death[93,117,120–125]. In the absence of constraints, parasites are expected to evolve an infinite transmission rate and zero virulence since this maximizes the infectious period. However, it is generally expected theoretically—and often found empirically—that increasing parasite transmission rate comes at the cost of higher virulence, resulting in premature host death that reduces the infectious period[125–132]. Under these constraints, parasites are expected to evolve intermediate to high, but not infinite, levels of virulence.

Beyond this basic 'trade-off model' of virulence evolution, studies have identified other regulators of virulence. For example, transmission opportunities are generally greater for parasites that are transmitted horizontally (between unrelated individuals) than for parasites transmitted vertically (from parent to offspring). As a result, horizontally transmitted parasites are expected to evolve higher virulence[117,133,134]. In addition, many hosts are co-infected with multiple strains of the same parasite species, and competition between these strains is expected to select for greater parasite virulence[93,122,135]. Increased horizontal transmission and within-host parasite competition are probably contributing to virulence evolution in honeybees, especially in managed systems. In modern beekeeping, the practice of replacing diseased with new susceptible colonies is also likely to have a major impact on the evolution of virulence. In this case, higher virulence increases the chance of the 'birth' of a new susceptible colony, in contrast to natural systems. This is clearly of benefit to the parasite, reducing the transmission cost of virulence and likely selecting for higher virulence. To determine how beekeeping results in virulence evolution by creating such novel demographics requires explicit theoretical models.

A necessary condition for virulence evolution is the existence of genetic variation in parasite virulence, which has been demonstrated in a number of honeybee parasites. Strains of the chalkbrood fungus *Ascosphaera apis* varied in larval mortality rates[136], strains of deformed wing virus caused different mortality in adult bees[137], and several studies are consistent with genetic variation in *Varroa* mite virulence[138–141]. No robust studies have been carried out on virulence evolution, but basic theoretical assumptions are likely to be met. For example, bee colonies with higher numbers of *Varroa* mites are more likely to collapse[25,26,28,29,53,142], probably as a result of direct damage inflicted by haemolymph-feeding mites and viruses vectored by the mite[25,28,143]. Higher *Varroa* loads in colonies increase the transmission of mites to other colonies through natural processes such as drifting (bees enter the wrong hive by accident), robbing (bees enter other colonies to steal food), and beekeeping practices such as movement of brood frames between hives[144]. Thus, the honeybee–*Varroa* system meets the theoretical assumption that greater mite exploitation of a bee colony results in both greater virulence and between-colony transmission.

Spatial structure of transmission. Beyond transmission rates, the spatial patterns of transmission also play a key role in virulence evolution. Theoretical models show that when transmission occurs across long distances, parasites tend to evolve higher virulence than when it occurs locally (that is, between near neighbours)[145–151]. This is due in part to three interacting processes when transmission occurs locally. First, parasite infection results in a shielding effect by which infected individuals are surrounded by other infected or immune individuals who can no longer become infected. Second, highly virulent parasites drive themselves to extinction by killing all hosts in a local spatial cluster. These linked dynamics mean that highly virulent parasites truncate their own transmission via the extinction of nearby susceptible hosts[151]. Finally, genetic correlations also contribute to the lower virulence of locally transmitting parasites[147,151–153]: when transmission occurs locally, parasites mostly compete with genetically related parasites for susceptible hosts, which is predicted to result in lower virulence.

Every year, American beekeepers generate conditions for increased virulence by routinely moving bees and brood (and their parasites) between colonies and by moving hundreds of thousands of colonies across the country for pollination contracts (Fig. 3c,d). Indirect evidence indicates that these practices have already resulted in more virulent parasites[154]. As one example, a Norwegian study found that colony losses tended to be higher among migratory than small-scale stationary beekeepers[155]. Moreover, in a cross-fostering study, Seeley[156] inoculated feral bees (with a history of surviving

Varroa) and managed bees (with a history of succumbing to *Varroa*) with mites from managed colonies, and found no differences in bee resistance, thus suggesting that feral colony survival may be due to mite avirulence. This is particularly relevant since feral colonies are characterized by much more local transmission than managed bee colonies (Fig. 3).

Host population heterogeneity and virulence evolution. Most virulence evolution models assume homogeneity in host populations when in reality they vary in parasite susceptibility. Models that account for this variation have found that host heterogeneity can maintain parasite polymorphism[157,158], and experimental studies have confirmed these predictions, for example in gypsy moth–baculovirus systems[157]. Host heterogeneity reduces specialization of parasites on any single host genotype, thereby reducing selection for a dominant virulent parasite genotype[159,160]. Thus, in addition to providing direct benefits in terms of reducing disease progression (discussed above)[70–72], genotypically diverse honeybee colonies are also less likely to select for highly virulent parasites.

Imperfect treatment and virulence evolution. It can seem paradoxical that selection for increased virulence can derive from actions taken to combat disease. As outlined above, parasites that reduce their own transmission by prematurely killing their hosts are not expected to be favoured by natural selection. However, theory has shown that disease treatments that act by reducing parasite growth—as opposed to preventing or curing infections—can remove this cost of high virulence, thereby retaining virulent parasites in the population[161–163]. When infecting untreated hosts, such parasites grow faster and thereby cause greater levels of disease. Similarly, treatment based on increasing tolerance—the ability of a host to maintain fitness without reducing parasite burden—can select for highly virulent parasites[164,165]. Empirical evidence has been shown in Marek's disease, a viral ailment of poultry, in which the use of imperfect vaccines—which reduce viral growth but do not prevent infection—has coincided with increases in virulence[166], and vaccinated chickens are able to transmit strains that are lethal to unvaccinated chickens[167]. Thus, in honeybees, the use of treatments such as acaricides that reduce parasite populations, but do not completely clear infestations, may similarly favour more virulent strains.

Cross-species transmission and virulence evolution. Some of the most devastating parasites are new to *Apis mellifera*. The original host of *Varroa destructor* is the Asian honeybee, *Apis cerana*[168]. Similarly, molecular studies have suggested that the microsporidian *Nosema ceranae* jumped species from the Asian honeybee to *Apis mellifera* in the past two decades[21,169].

New host colonizations can have important consequences for host survival and parasite evolution[170]. Emerging parasites, such as SARS, HIV and Ebola viruses in humans, are often highly virulent. Such high virulence can arise through at least two processes. First, theoretical studies show that when parasites jump to a novel host species, they encounter a fully susceptible host population, which increases transmission opportunities and thereby selects for increased virulence[127,171–174]. Such increased transmission opportunities probably underlie the observed increases of virulence of *Mycoplasma gallisepticum*, which has recently emerged in house finch populations in North America[175]. Second, natural host-parasite interactions result in coevolutionary dynamics whereby hosts evolve tolerance or resistance as parasites evolve virulence[165,176,177]. Such highly evolved parasites may express much greater levels of virulence when they infect a novel host species without such resistance or tolerance mechanisms.

Both of these processes are likely important in emerging diseases of honeybees. For example, although *Varroa destructor* causes significant morbidity and mortality in *A. mellifera*, *Apis cerana* colonies
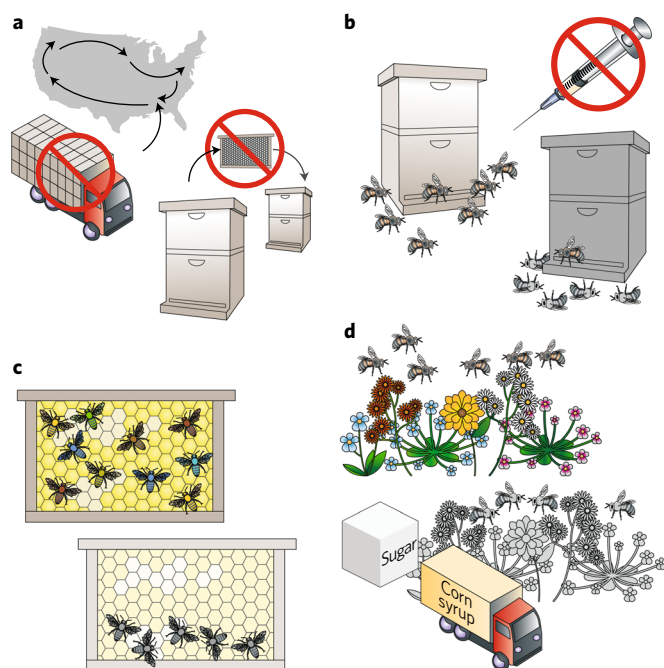
Fig. 4 | Management applications. a, Transmission reduction is a key management goal to reduce both ecological disease pressure and selection for greater parasite virulence; transmission reduction should occur at multiple scales, including the two shown here: between colonies within an apiary, and at continental scales. **b**, Promoting 'survivor stock', that is, allowing colonies with low parasite resistance to naturally die can increase the evolution of honeybee resistance. **c**, Increased colony-level genotypic diversity improves disease outcomes and supports general colony health, and may also reduce selection pressure for increased virulence evolution. **d**, Increased dietary diversity and reduced dependence on processed sugars can support better bee health and disease resistance, both for individual bees and for group-level defences.

are generally infested without measurable colony-level effects. This is partly because *Varroa* only infests drone (male) brood in *A. cerana*[138,178–180], and *A. cerana* possesses behavioural defences to resist the mite, including removal of infected brood and social grooming. In *A. mellifera* colonies, *Varroa* is not limited to male brood, and its exploitation of worker brood (which comprise the vast majority of brood cells in a colony) allows for much greater population growth. In another example, the recent host shift of *Nosema ceranae* to *A. mellifera* has resulted in virulence in its new host; however, another microsporidian, *Nosema apis*, known to have infected *A. mellifera* for more than a hundred years, is much less virulent[181], which supports the idea of attenuated virulence in the more coevolved relationships.

In natural populations, increases in adaptive virulence are expected to be transient[127,171–174]. Once a parasite has spread throughout the novel host population, the availability of susceptible hosts is reduced, selecting for lower parasite virulence. This process is supported by theory[127,171–173] and empirical studies[173,182]. Within a few years after releasing a hypervirulent strain of myxoma virus to control rabbit populations in Australia, the virus evolved lower virulence, which maximized its between-host transmission[183]. Continued infection dynamics also select for more tolerant and resistant hosts, thereby reducing expressed virulence. This does not mean, however, that honeybee diseases will become less virulent in years to come. Instead, current beekeeping practices artificially sustain high transmission levels and also prevent the evolution of honeybee resistance or tolerance by employing artificial disease

control[57]. Thus, without changes in current beekeeping practices, we should expect continued selection for highly virulent parasites.

Applications

The importance of ecological and evolutionary approaches to disease control is underscored in other agricultural systems. As described above, routine vaccination of poultry against Marek's disease has resulted in the evolution of virus hypervirulence[167]. Additional factors leading to high virulence in this virus include high chicken rearing densities and shorter cohort durations achieved by selective breeding[166]. Short cohort duration is expected to select for enhanced virulence because the cost of virulence (truncation of transmissible period) is less severe in short- versus long-lived hosts[159]. Similarly, high fish densities and fast maturation may have contributed to increased virulence evolution in aquaculture[159].

We began this Review by discussing host density, a key variable in disease ecology. Host density was directly managed in the 2001 foot-and-mouth disease outbreak in the UK, where pre-emptive culling of farms nearby infected farms was effective in reducing the spread of the disease[184]. Similarly, in sugar beet systems, models show that crop removal in farms adjacent to farms with rhizomania disease could effectively reduce disease spread[185]. Both strategies essentially reduce the local density of susceptible 'individuals' (farms), thereby reducing parasite transmission.

We can apply lessons from disease ecology and evolution to improve parasite outcomes in honeybees. We distil these to six concrete management suggestions, which are specific to the biology of honeybees and the management context of beekeeping (Fig. 4). We then discuss the trade-offs between ecologically and evolutionarily minded strategies with other management goals.

Reducing disease transmission. Transmission reduction has potential benefits in terms of ecology (reducing the prevalence, severity and duration of outbreaks) and evolution (reducing selection for higher virulence). In honeybees, transmission occurs at multiple hierarchical scales, including within colonies; between colonies within apiaries; between apiaries; and within or between regions, countries, and continents. The modular and standardized feature of hive designs encourages transmission at all these scales. At the apiary level it is routine—even recommended—that beekeepers 'equalize' colony strength by moving combs of brood between colonies. At regional and national scales, hundreds of thousands of beehives are moved for pollination and honey production. In the United States, over half of all honeybee colonies are rented annually for almond pollination in California. This mass transport and mixing of bees is likely to be a major contributor to parasite transmission. Transmission reduction at such spatial scales is a matter for policy-level regulation, as has recently been called for by the IPBES pollinator report[186], though with a focus on limiting spread of new disease agents rather than a focus on virulence evolution.

Improving disease treatments. Improved disease treatments are a longstanding desire of beekeepers[187], but disease evolution theory[162,163] and evidence from Marek's disease[167] highlight that we should strive to develop treatments that provide complete parasite clearance, in contrast, for example, with most means of *Varroa* mite control which only reduce mite numbers in a colony. Such incomplete treatments—and similarly, management techniques that promote parasite tolerance over clearance—increase selection for parasite virulence[164,165].

Using survivor stock. The idea behind survivor stock is to 'let nature takes its course' and allow sick colonies to die, thus propagating only surviving hosts. It constitutes the flip side of the coin from virulence reduction: increasing host resistance. Keeping susceptible bees alive through multiple interventions—the approach typically taken in beekeeping—dilutes natural selection for disease

resistance. In contrast, allowing disease survivors to propagate and (importantly) allowing nonresistant colonies to perish could lead to more resistant bees with fewer disease problems. Keeping the sickest bees alive has the same effect as a leaky vaccine or high disease tolerance: it reduces the cost of virulence, and as such may lead to the evolution of more virulent parasites. In the case of the parasitic *Varroa* mite, there is evidence that a survivor stock approach has succeeded in producing bee stocks that do not require routine intervening treatments[188,189]. There are, however, short-term risks with this approach that should be anticipated: dying colonies often have high drifting rates, potentially increasing parasite transmission between colonies[62].

Increasing genotypic diversity. Increasing genotypic diversity is another tactic for improving disease resistance. In most other agricultural contexts, greater genotypic diversity may save some individuals in a flock, herd, or field. But in honeybees, the colony is the unit of selection, and greater within-colony genotypic diversity—due to polyandry (multiple mating) in honeybee queens—bolsters individual colonies. Colonies from queens with higher mating number have greater disease resistance and overall survivorship[70–72,190]. Genotypic diversity can be considered at scales beyond the colony. For example, the survivor stock approach discussed above may encourage disease resistance diversity at a landscape scale, assuming the presence of a variety of resistance alleles in breeding populations.

Managed honeybees have been bred to express resistance traits. Chief among these are *Varroa*-sensitive hygiene, the phenotype by which nurse bees detect *Varroa*-infested brood and remove them from the colony[191,192] and auto- or allo-grooming in which workers remove mites off their own or nestmates' bodies[193]. A key challenge is that trait-breeding approaches operate in apparent tension with the simultaneous pursuit of within-colony diversity. Classical animal breeding, including in honeybees, is based on reducing overall genotypic diversity (for example, through back-crosses or closed populations) in order to increase incidence of targeted alleles[194,195]. Maintaining such alleles in managed bees is difficult given large dispersal distances of drones[196], queen multiple mating, and high labour demands for maintaining inbred lines, all of which contribute to the relatively low adoption of *Varroa*-sensitive hygiene and other selected traits among beekeepers[197]. More work is needed to understand how: (1) genotypic diversity can be tractably maintained or increased by beekeepers operating in different management contexts; and (2) if and how genotypic diversity can be integrated into traditional breeding programs that have successfully identified resistance alleles.

Supporting behavioural resistance. It is critical that beekeepers support honeybee behavioural defence mechanisms and other forms of social immunity. This can be challenging when these mechanisms conflict with other beekeeping interests. One example is the long-standing bias in American beekeeping against propolis, which is known to increase colony-level resistance to several parasites[198] but which also 'gums up' hives, making it difficult to open hives and separate hive components. Second, beekeepers routinely manage colonies to discourage reproductive swarming, but it is now known that swarming temporarily reduces both the adult bee population and available brood, thus negatively impacting tracheal mites[199] and *Varroa*[54]. There are near-term evolutionary ramifications to these conflicts, and beekeepers may have selected for bees that are more convenient to manage but handicapped in behavioural defences. More work is needed to identify other behavioural and social immune pathways and integrate them into profitable beekeeping practices.

Increasing dietary diversity and flexibility. A related intervention is reducing honeybee dependency on processed sugars and instead providing them with a varied floral diet. In addition to optimizing individual bee health and immune function, plant diversity supports behavioural and social immune defences, for example by providing bees taxonomically rich sources of propolis[85,198,200] and promoting production of hydrogen peroxide in honey[79]. In addition, certain bacteria in the honeybee gut microbiome help reduce disease burdens. Access to greater diversity of flowering plants allows honeybees to acquire or maintain such beneficial microorganisms[115].

Management trade-offs. We cannot understate that all of these management suggestions are most difficult to implement in those systems that have the most to gain from them. Integrating these biology-based practices into commercial-scale migratory beekeeping requires translational research to identify specific actions that are practical and profitable. It will require the work of social scientists and educators to modify human behaviours. Change requires that management for disease be accomplished with actions that are affordable and economically and socially beneficial for beekeepers. Despite thin profit margins, we hope that the prospect for realized sustainability will be powerful motivation.

In addition, buttressing the health of managed pollinators must not be done at the expense of native pollinators, as honeybees can serve as reservoirs of diseases that threaten wild bees[201–203] and other managed bees[204]. More than 20,000 species of wild bees play critical roles in agriculture and native ecosystems, and disease risk is a threat to this critical component of biodiversity[10,186]. Many of the disease management interventions we suggest could benefit native bees by reducing disease levels in honeybees, thus reducing the risk of spillover to other species. But others—such as providing honeybees access to diverse forage plants—could increase contact between species, thus increasing spillover risk. This may be especially true when forage plant density is low, thus forcing managed and wild bees to share plants more often. More research is needed to understand the risk of parasite spillover, as well as the full range of trade-offs associated with different honeybee disease management interventions.

Outlook

Although further research on honeybee disease ecology and evolution is critical for beekeeping and pollinator-dependent agriculture, such work can contribute to our understanding of disease ecology and evolution at fundamental levels, given several particulars of honeybee natural history. For example, honeybees are eusocial with a hierarchical population structure[205], thus forming a system distinct from non-social organisms on which evolutionary and ecological pressures may operate distinctly. Second, honeybee colonies are found in varying densities and in both stationary and migratory populations, constituting an attractive model system for exploring the role of spatial structuring in virulence evolution.

Studying honeybee diseases comes with logistical challenges in terms of experimental design and execution. A key issue is replication, and for most applied disease studies, the unit of an independent sample is apiary—not colony—as apiary-level effects are well described in the beekeeping literature[206]. Managing apiaries that are separated in space, with sufficient numbers for adequate sample size, is expensive and represents a logistical challenge. Because of these challenges, most previous work in this realm has unfortunately not been replicated at the apiary level. Second, in most temperate climates honeybees have a distinct annual cycle that contributes variation to key colony parameters such as adult population size, brood production, and foraging. Studies focused on disease ecology and evolution must work within that annual cycle: if experimental manipulations fail once, they cannot be repeated until the following year. Third, honeybee colonies display variation even in closely related colonies within the same apiary, in terms of size, brood production, queen mating number, and temperament. Many of these parameters have direct bearing on responses to disease threats. Thus, it is critical that

disease studies standardize both honeybee colonies and parasites as much as possible. For example, studies can start with replicated apiaries with nucleus colonies with the same hive body design and equivalent numbers of workers and new queens that are genetically related. Fourth, disease studies should inoculate colonies with carefully measured, identical infectious doses. As with apiary-level replication, most disease studies in honeybees do not meet these criteria. Given the need for such studies, we suggest that funding agencies recognize these unique and challenging design needs and consider increasing funding limits for honeybee disease research proposals.

Conclusion

Parasites form the greatest threat to the most economically important managed pollinator in the world. It is critical that we aim for health management practices that are rooted in the fundamental principles of evolution and ecology. To our minds, it is indefensible that current beekeeping practices are not only predicted to create more severe outbreaks, but to select for greater virulence. Developing new management systems that recognize ecological and evolutionary processes and constraints will take a global interdisciplinary effort uniting scientists from many fields with beekeepers and farmers. Such an effort must accommodate the challenging particularities of research on honeybees, but doing so will contribute to fundamental understanding of systems-level disease processes to the benefit of all. Such management systems must address other stressors, in particular pesticides and other agrochemicals known to negatively impact honeybees and wild bees[18,207–210]. Fully accounting for the evolutionary and ecological contexts of disease is a foundational step toward maintaining the agricultural productivity and security upon which a growing human population depends.

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Competing interests

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