Reduced parasite burden in feral honeybee colonies

Patrick L. Kohl[1,*], Paul D'Alvise[2,§], Benjamin Rutschmann[1,3], Sebastian Roth[3], Felix Remter[3,4], Ingolf Steffan-Dewenter[1] & Martin Hasselmann[2]

[1] Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Würzburg, Germany

[2] Department of Livestock Population Genomics, Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

[3] BEEtree-Monitor, Munich, Germany

[4] Katholische Stiftungshochschule München

[§] Current address: Institute for Medical Microbiology and Hygiene, University Hospital Tübingen, Tübingen, Germany

[*] Correspondence; email: patrick.kohl@uni-wuerzburg.de

Abstract

Parasites are the main threat for managed honeybee (*Apis mellifera*) colonies. It is unknown whether feral colonies that escaped from apiaries benefit from parasite-reducing mechanisms like swarming or suffer from high parasite pressure due to the lack of disease management. We compared the occurrence of 18 microparasites among managed (N=74) and feral (N=64) colony samples from Germany using qPCR. We distinguished five colony types representing differences in colony age and management histories, two variables potentially modulating parasite prevalence. The number of parasite taxa was lower in feral colonies (mean: 5.4) than in managed colonies (mean: 6.1). Parasite community compositions also differed, with Trypanosomatidae, Chronic bee paralysis virus, and Deformed wing viruses A and B being less prevalent and abundant in feral colonies. Parasite burden was lowest in newly founded

feral colonies, intermediate in overwintered feral colonies and managed nucleus colonies, and highest in overwintered managed colonies and hived swarms. Our study confirms the hypothesis that swarming temporally reduces parasite pressure in feral honeybee colonies. Furthermore, differences in the environment between wild nests and hives at apiaries must be involved in explaining the observed differences. We conclude that feral honeybees are unlikely to contribute significantly to the spread of bee diseases.

Keywords

Bee diseases, disease management, horizontal transmission, pathogen spillover, swarming, wild honeybees, *Varroa destructor*, viruses.

Introduction

Honeybees are social insects that live in large perennial nests, and as such they are naturally attractive hosts for a range of parasites including arthropods, fungi, bacteria, and viruses [1]. However, parasites are thought to rarely limit their populations under natural conditions [2–5]. Thanks to a range of defence behaviours referred to as “social immunity” [6], e.g., the eviction of infected brood [7] or the application of antimicrobial plant resin within the nest [8], honeybee colonies can naturally keep many disease-causing agents in check. Furthermore, parasite populations are thought to be regulated within colonies as a side-effect of the natural cycle of colony reproduction and dispersal [9,10]. Colonies reproduce via fission, when the old queen and approximately 70% of the workers leave as a swarm to build a new nest in another cavity [11]. The swarming bees do not take any brood with them, and it takes around three weeks until a young queen resumes egg-laying in the old nest; hence, brood production is interrupted in both colony parts and the reproduction of parasites infecting the brood or young workers is halted [9,12].

Under modern rationalized beekeeping, honeybees' disease ecology is changed on various scales [13]. By preventing swarming and by providing spacious hives beekeepers maintain unnaturally large, continuously breeding colonies, thereby increasing the habitat available for parasites within colonies [9]. Crowding colonies in apiaries facilitates the transmission of parasites between nests [14–16], and the movement of combs in frames or whole hives promotes parasite transmission between apiaries [17]. On a global scale, the large-scale transport of colonies can expose the bees to entirely novel parasites [18]. The worldwide invasions of Western honeybee (*Apis mellifera)* populations by the ectoparasitic mite *Varroa destructor*, whose natural host was the Eastern honeybee *A. cerana*, famously demonstrated the potentially devastating effect of host-switches [19]. The mites acted as a new vector of previously rare Deformed Wing Viruses which are now killing colonies worldwide [20]. Given these epidemic promoting factors, it is not surprising that parasites are regarded as the most important threat to managed honeybees today, and that monitoring disease and controlling parasites are among the main activities of beekeepers [13,21].

When the bees have no evolved defences, novel parasites are also likely to threaten wild-living honeybees. It is well documented how the arrival of *V. destructor* led to an initial decline in feral populations of temperate-adapted Western honeybees in the USA [22,23]. However, populations recovered within only a few years, probably at least partly because natural selection gave rise to resistance or tolerance mechanisms [24–26]. Unfortunately, the situation is different in regions where most honeybee colonies are managed and where feral colonies are merely recent escapees from apiaries, as is the common situation in Europe [27,28]. Where feral colonies directly descend from managed colonies, they are equally naïve towards the parasites. Consequently, in the absence of treatment by beekeepers, they might develop high parasite loads [29]. However, there are also arguments for why feral colonies might still have, on average, relatively low parasite loads. In Germany, about 90% of feral

colonies present in summer are young colonies recently founded by swarms [28], and these probably start their colony cycle with low infestation levels [12,30]. Furthermore, the rate at which new parasite species enter feral colonies should be reduced in comparison to the situation at the apiary because their nests are typically widely dispersed in the landscape [14,15,31].

Understanding the disease ecology of recently feralised honeybee colonies is important because they potentially create a management conflict: under European legislation, honeybees are considered livestock animals and beekeepers are obligated to register their hives, regularly apply and document miticide treatments, and report infectious diseases to prevent epidemics. If feral colonies carry high loads of parasites, however, they might reinfect managed colonies, thereby undermining the veterinary measures undertaken to combat disease [29,32]. When highly infected feral colonies disperse into natural areas, their parasites might also spill over to, and harm, populations of other (wild) bee species [33–35].

To assess whether feral honeybees pose a risk to the health of managed and wild bee populations, we compared the occurrence of 18 microparasite taxa in feral and managed honeybee colonies using qPCR [36]. We collected colony samples from four regions in southern Germany encompassing both rural and urban landscapes, to cover cases from different environments with potentially different parasite communities [37]. By taking all samples within a four-week time window in July, we made sure that variation in parasite communities could not be attributable to seasonal variation [36]. We chose this point in time because it is epidemiologically relevant: a high frequency of between-colony robbing behaviour due to nectar scarcity in summer [38] increases the risks of parasites transmissions, and parasite loads in summer affect subsequent colony winter survival [39]. Lastly, in addition to the difference between managed and feral we distinguished five colony types representing different colony age classes and management histories, two factors that

potentially modulate parasite prevalence. The collected data allowed us to make a nuanced assessment of the effect that the “managed” and the “feral” environment have on the parasite prevalence in honeybee colonies.

Material and Methods

Sample collection

We collected worker bees from managed colonies (N=74, from 73 apiaries) and feral colonies (N=64, from 63 sites) in four regions in southern Germany (Swabian Alb, counties Coburg and Lichtenfels, county Weilheim-Schongau, and the city of Munich; Figure 1) in July 2020. Information about nest sites of feral colonies had been obtained before during population-monitoring studies ([28]; S. Roth and F. Remter, unpublished), and managed colony locations and the respective sampling permissions were obtained by contacting local beekeepers (see supplementary information for details). Approximately 20–50 bees per colony were captured by placing a white butterfly net in front of the hive or cavity entrance. The bag of the net was permanently stretched out with a metal bow so that bees leaving the nest would directly fly in. To reach the nest entrances of feral colonies we prolonged the handle of the net with a telescopic rod (see supplementary information in [28]). With this method, we primarily sampled foragers on their outbound flights. The sampled bees did not show any overt disease symptoms. After capture, they were directly freeze-killed and collected in 50 mL vials. They were permanently kept on dry ice during transport and stored in freezers at -80°C until the analyses.

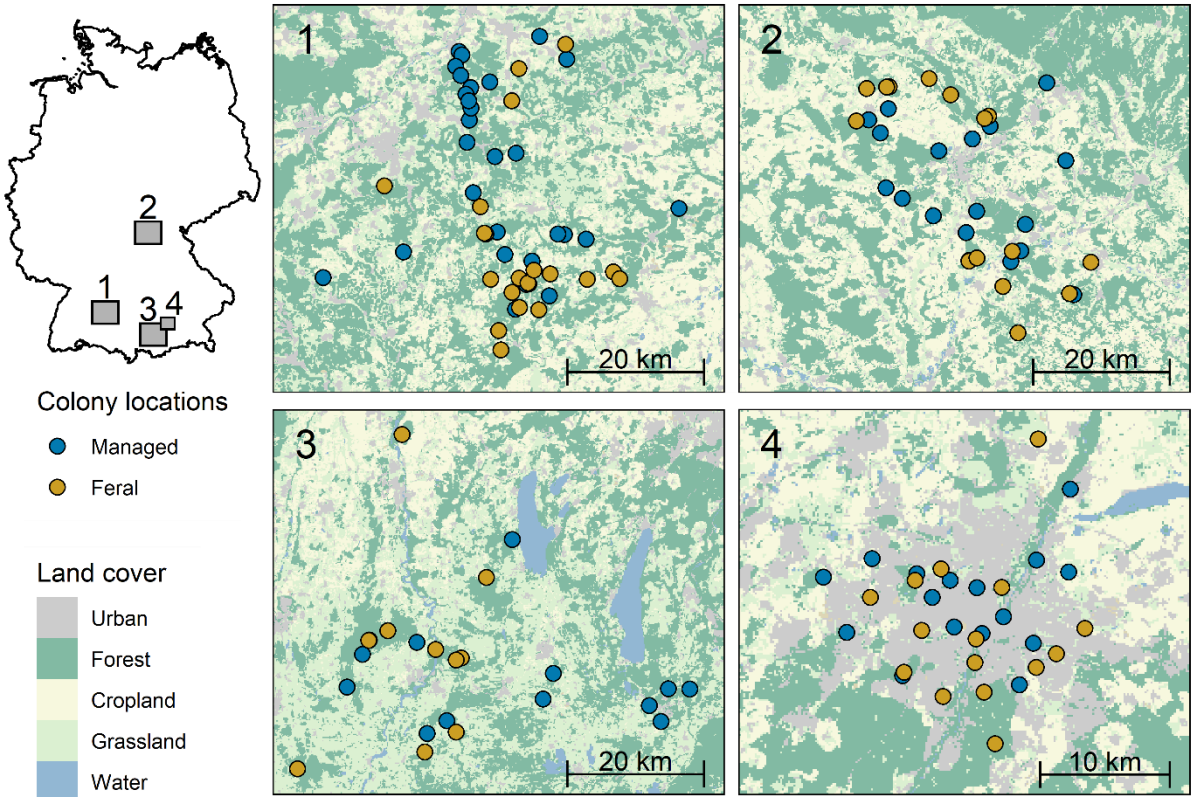

Figure 1. Geographic locations of the sampled managed (N = 74) and feral (N = 64) honeybee colonies in four regions in southern Germany (1: Swabian Alb, 2: Counties Coburg and Lichtenfels, 3: County Weilheim-Schongau, 4: City of Munich). Note that the map of Munich has a different scale. Land cover data are from [40].

Assessing colony age and management history

For each sampled colony, we noted whether it had overwintered at least once (age > 1 year), or whether it had been newly founded in the year of sampling (age < 1 year). In the case of feral colonies, we knew the recent history of all nest sites, and in the case of managed colonies, we obtained this information from beekeepers. Among feral colonies, 72 % (N=46) were recent founders (swarms that moved into the cavity and founded a colony in the spring swarming season of 2020), and 28% (N=18) had overwintered at least once. Among managed colonies, we sampled similar numbers of overwintered (N=38; 51%) and newly founded colonies (N=36; 49%), since we assumed this would well represent the ratio of old versus young colonies at apiaries in summer. In the group of young managed colonies, we further distinguished between nucleus colonies, i.e., daughter colonies created by taking frames with

brood and bees from an established colony (N=27) and hived swarms (N=9). The latter were either natural swarms captured by beekeepers (N=7) or man-made swarms ("packages") created by brushing bees off the combs of a mother colony and putting them into a new hive together with a queen (N=2).

Parasite testing

We assessed the colony-level occurrence of 18 microparasites using high-throughput qPCR on a Biomark HD system (Fluidigm, San Francisco, CA) with parasite-specific primers [36,41–47] closely following an established protocol [36], with the exception that RNA was extracted from homogenates of 20 workers per colony, not from individual bees (see supplementary information for details on RNA extraction and Table S1 for a list of the parasites and reference genes assayed). We ran three qPCR replicates for each assay and considered a parasite taxon as present in a colony if target molecules were detected in at least two. If a colony sample was positive, we averaged the number of target molecules detected in all three assay replicates to obtain a mean number of target molecules per colony sample. As a measure of colony-level parasite abundance, we calculated for each parasite the number of detected target molecules per 100 ng of extracted RNA and reported the logarithm of this ratio, $\log_{10}$(n/100 ng RNA+1), as the "parasite load".

Statistical analyses

All statistical analyses were performed with R version 4.0.5 [48] and figures were created using "ggplot2" [49]. We analysed whether the number of detected parasite taxa per colony (univariate analysis, count data) and the microparasite community composition (multivariate analysis) differed depending on "management" (two levels: "managed" versus "feral") or "colony type" (five levels: "managed: overwintered", "managed: nucleus colonies",

“managed: hived swarms”, “feral: overwintered” and “feral: founders”). In all analyses we accounted for the potential effect of spatial location on microparasite communities.

We analysed the number of parasite taxa detected using generalized linear models with generalized Poisson distributions (function “glmmTMB”; [50,51]). We included “study region” (four levels) as a fixed factor representing the spatial structure of the data. This was reasonable since most spatial variation in parasite assemblages was attributable to variation between regions rather than variation between sites within regions (see below). Model predictions of the mean number of parasite taxa and 95% confidence limits were produced using the function “emmeans” [52], and statistical significance was assessed using likelihood ratio tests (LRT), the effect of management or colony type being assessed after accounting for regional differences (e.g., “anova(model 1: Number of parasites ~ region, model 2: Number of parasites ~ region + management)"). In the case of the five-level factor “colony type”, we used the function “glht” from the “multcomp” package [53] for Tukey post hoc tests of pair-wise differences in parasite number between colony types.

To analyse differences in parasite community composition, we used distance-based redundancy analyses with management as the constraining factor (dbRDA, function “db.rda” from the “vegan” package, [54]). Redundancy analysis summarizes multi-factor variation so that dissimilarities between samples can be graphically displayed in a two-dimensional coordinate system whose axes best separate the data based on predefined factors of interest (“constrains”). We chose to analyse parasite community compositions based on Jaccard distances (and thus based on presence/absence of parasite taxa) as opposed to Euclidean distance or Bray-Curtis dissimilarities (based on colony-level parasite abundance), because abundance data contained many zeroes and were non-normally distributed, and because it is hard to compare target molecule abundance variation of several orders of magnitude between parasites as different as arthropods and viruses. To partial out spatial structure, we

conditioned our dbRDAs on principal coordinates of neighbour matrices (function "pcnm" from the "vegan" package; [54,55]). This revealed that spatial location explained 17.7 % of the variation in parasite communities. Interestingly, a test with "study region" (four levels) as a constraining factor showed that 14.9 % of the variation could be attributed to differences between the four regions (see Figure S1). This means that the spatial structure in parasite communities was mostly caused by large-scale (between regions) rather than fine-scale (between sites within one region) spatial variation. To infer the statistical significance of the constraining factors we used permutation tests with 99999 permutations ("anova.cca" function).

We did not perform separate statistical tests for each microparasite since potential interactions between taxa (both positive and negative) lead to non-independent data, and since the high number of individual tests would introduce statistical problems related to multiple testing. However, we graphically compared for each tested microparasite the prevalence and the associated 95% confidence intervals [56,57] as well as the mean colony-level loads.

Results

The number of microparasite taxa detected per colony ranged between 1 and 9, with an overall average of 5.8 taxa. Parasite counts differed significantly between regions (LRT: D.f.=3, χ^2= 23.294, P=0.00004); they were lowest in Coburg and Lichtenfels (mean: 4.9), intermediate on the Swabian Alb (mean: 5.8), and highest in Weilheim-Schongau (mean: 6.3) and Munich (mean: 6.2). On top of regional differences, management significantly affected the number of parasites per colony (LRT: D.f.=1, χ^2= 11.893, P=0.0006). Feral colonies had, on average, one parasite less (median: 5, mean: 5.4, range 1–8) than managed colonies (median: 6, mean: 6.1, range 4–9) (Figure 2a).

The distance-based redundancy analysis revealed a marginal difference in the composition of parasite taxa between managed and feral colonies (permutational anova: P=0.074), with management explaining 1.1% of the variation in microparasite communities (Figure 2b). A direct inspection of each of the 18 microparasites showed that prevalence was either similar in managed and feral colonies or lower in feral colonies (Figure 3). A clear reduction in prevalence of more than 10 percentage points was found in four microparasite taxa: *Crithidia/Lotmaria* (Managed: 90.5%, Feral: 78.1%), Chronic bee paralysis virus (Managed: 14.9%, Feral: 1.6%), Deformed wing virus A (Managed: 18.9%, Feral: 3.1%), and Deformed wing virus B (Managed: 47.3%, Feral: 35.9%). Comparing mean colony-level parasite loads yielded a similar result, with loads tending to be lower in feral colonies (Figure 4a). Considering only colonies which were tested positive showed that parasite loads of infected feral and managed colonies were generally similar (Figure 4b).

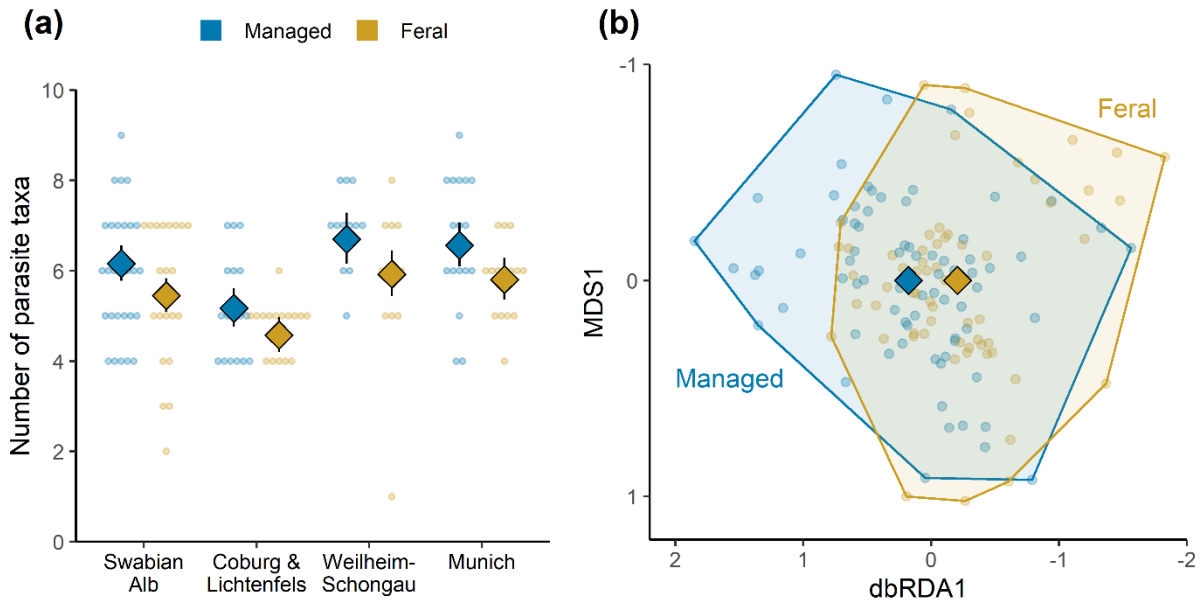

Figure 2. Comparison of parasite burden between managed (N=74) and feral (N=64) honeybee colonies. **(a)** Number of microparasite taxa detected among the 18 taxa assayed for each of the four study regions. Dots are raw data; diamond symbols and vertical lines give model-estimated means and 95% confidence intervals. **(b)** Representation of Jaccard dissimilarities in microparasite communities as created by a distance-based redundancy analysis with management as the constraining factor (spatial structure partialled out). Managed and feral colonies are separated along the dbRDA1-axis (1.1% of total variation).

The first unconstrained axis (MDS1) explains 16.7% of the variation. Dots represent locations of individual colonies and diamonds are mean locations.

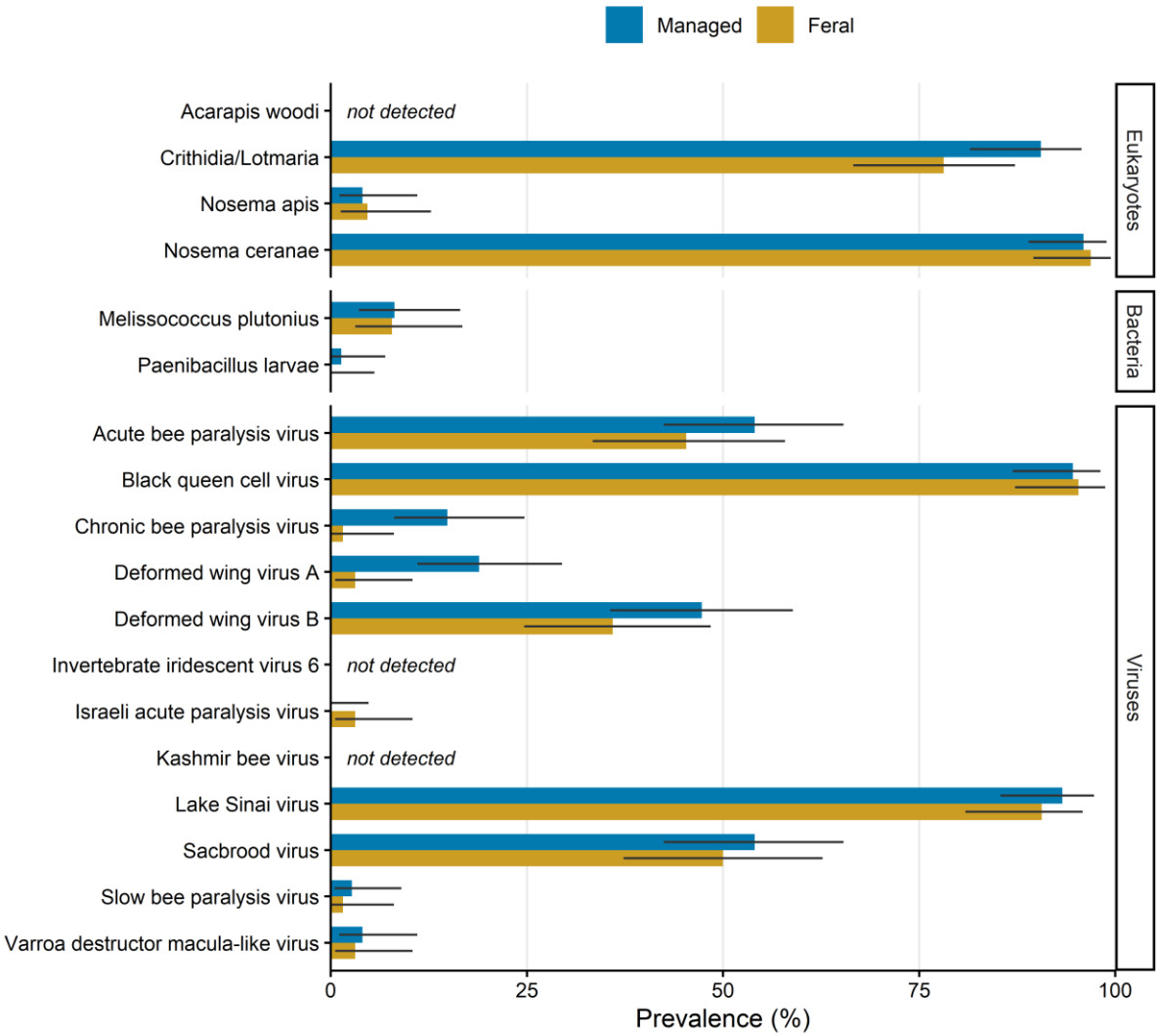

Figure 3. Prevalence (percentage of colonies tested positive) and 95% confidence intervals for 18 microparasite taxa among managed (N=74) and feral (N=64) honeybee colonies. See Table S2 for an overview of prevalence values.

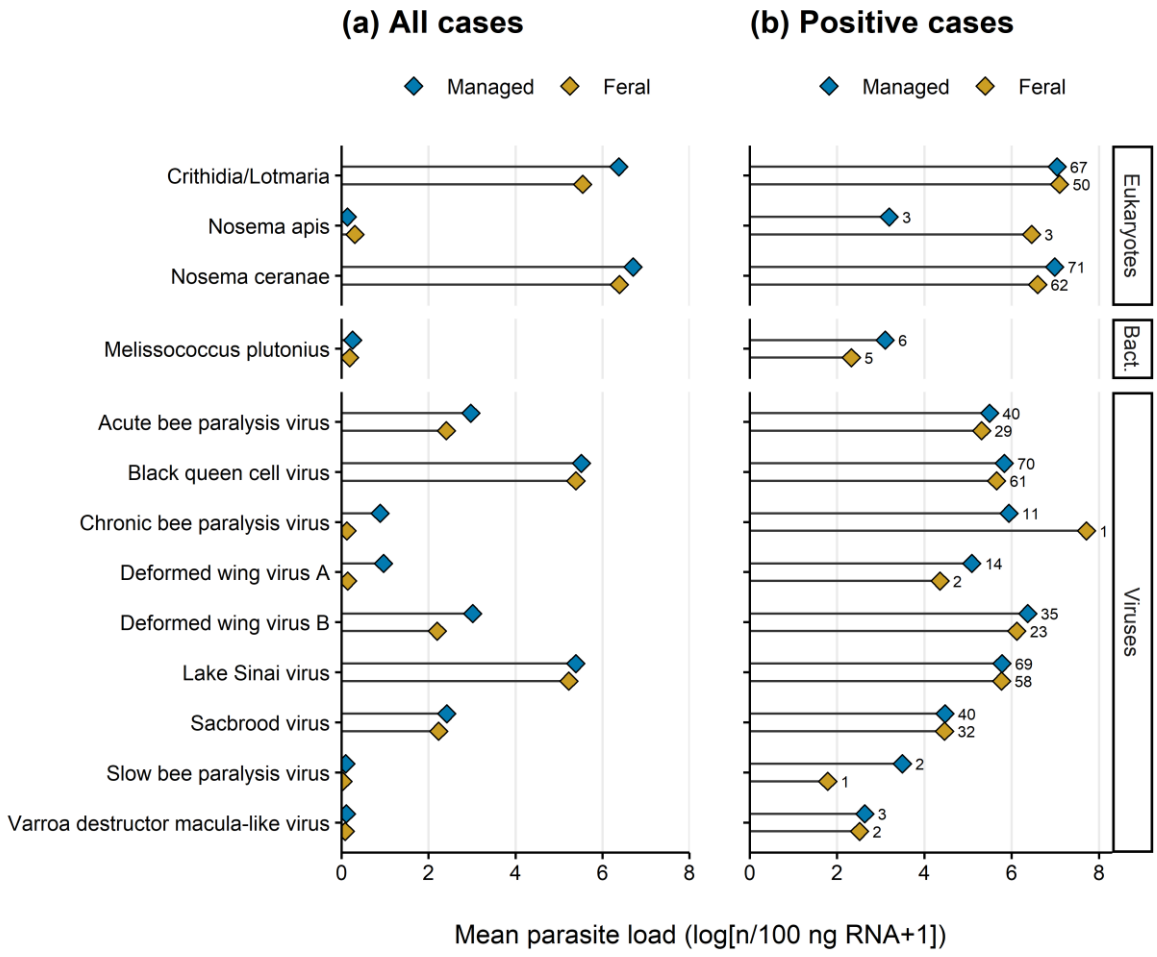

Figure 4. Mean parasite loads of managed and feral honeybee colonies. Shown are only microparasite taxa detected in at least one colony per group. **(a)** Mean parasite loads based on all colonies tested (managed: N=74, feral: N=64). **(b)** Mean parasite loads based on positive cases only (sample sizes are provided inside the plotting area). See Table S2 for an overview of parasite load values.

An analysis of the number of parasites in relation to five colony types gave a more nuanced picture of differences in parasite burden (LRT, "colony type" after "region": D.f.: 4, χ^2=19.719, P=0.0006). Parasite counts were lowest in newly founded feral colonies (mean: 5.3, range: 1–7), intermediate in overwintered feral colonies (mean: 5.7, range: 4–8) and nucleus colonies (mean: 5.7, range: 4–8), and highest in hived swarms (mean: 6.6, range: 4–8) and overwintered managed colonies (mean: 6.3, range: 4–9) (Figure 5a). The pairwise differences between feral founders and overwintered managed colonies (P < 0.001) and between feral founders and hived swarms (P = 0.0076) were statistically significant. Plotting the five colony types in the coordinate space created by the redundancy analysis with

“management” as the constraining factor revealed similar relative differences. Along the dbRDA1-axis, feral founders were separated from hived swarms and overwintered managed colonies, while overwintered feral colonies and nucleus colonies were in the centre (Figure 5b). This means that the parasite communities of feral founders, rather than those of overwintered feral colonies, represented “typical feral” communities, and that those of overwintered managed colonies and hived swarms, but not those of nucleus colonies, represented “typical managed” parasite communities. Accordingly, mean colony-level loads tended to be lower in feral founders compared to overwintered feral colonies, and among the three types of managed colonies, mean parasite loads were most often lowest in nucleus colonies (Figure 5c).

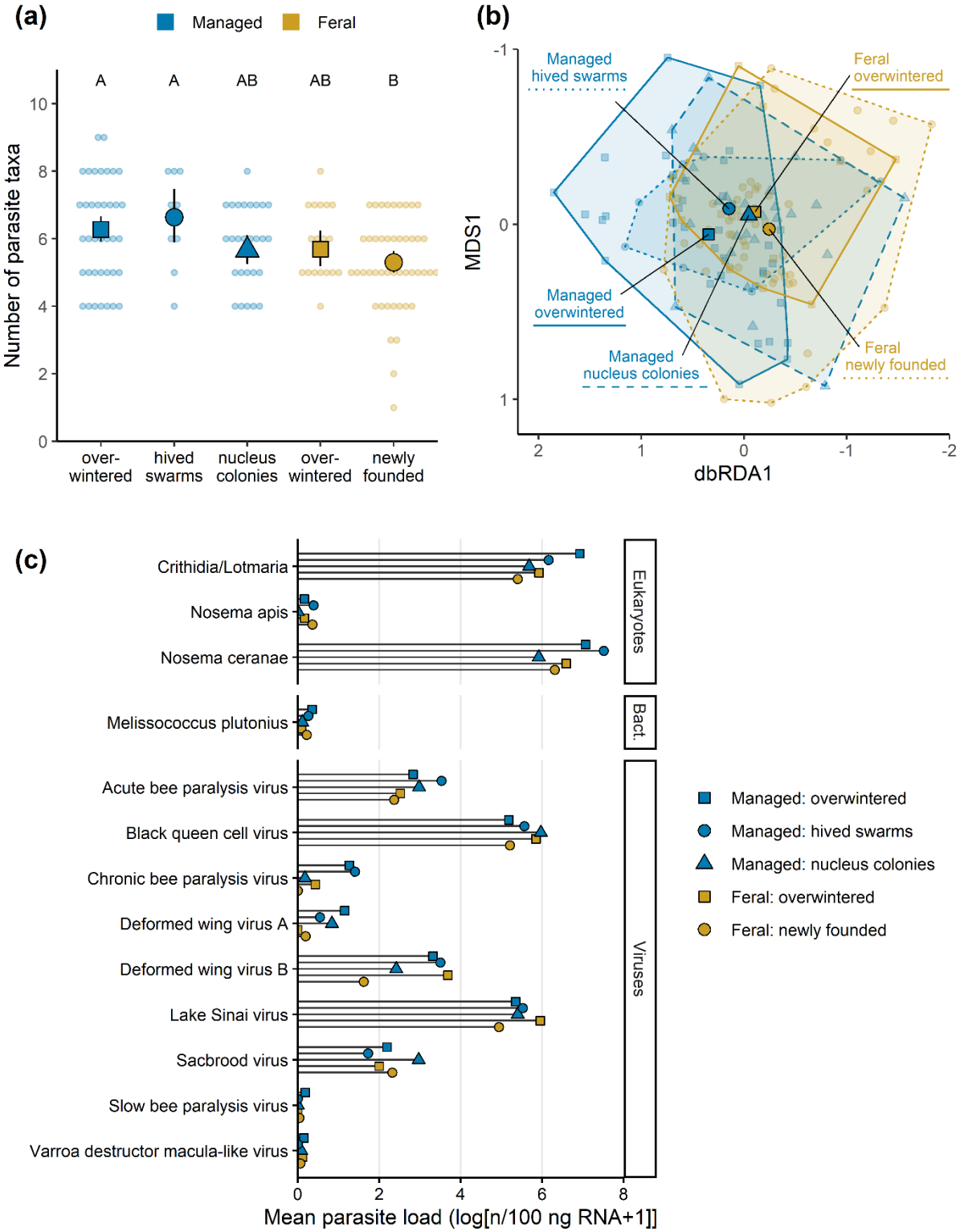

Figure 5. Comparison of parasite burden between different types of managed and feral honeybee colonies **(a)** Number of microparasite taxa detected among the 18 taxa assayed. Dots are raw data; large symbols and vertical lines give model-estimated means and 95%-confidence intervals. Pairs that do not share a letter differ significantly (P<0.05). **(b)** Mean parasite community locations of the five colony types (large symbols) in the coordinate space created by the distance-based redundancy analysis with management as the constraining

factor (same axes as shown in Figure 2b). Small symbols are individual colony locations and large symbols are mean locations. **(c)** Mean parasite loads. Only microparasite taxa detected at least once in each group are shown.

Discussion

We compared the parasite burden of honeybee colonies in managed and feral condition to evaluate whether colonies that escaped from apiaries pose a risk to bee health by acting as reservoirs of disease-causing agents. The number of microparasites detected per colony and the prevalence of four important taxa were clearly lower in feral compared to managed colonies. This was explained by differences in population demography, with most feral colonies being recent founders with very low parasite loads, and by environmental differences between managed and feral colonies. We conclude that feral honeybee colonies are unlikely to contribute disproportionately to the spread of bee parasites.

Although the region of sampling had a relatively stronger effect on the number of parasite taxa detected and on the composition of parasite communities per colony than management, we believe that the differences we found between feral and managed colonies are ecologically relevant. With about six parasite taxa detected per colony on the overall average, the reduction of nearly one parasite per colony from managed to feral colonies is noteworthy, especially since this pattern was consistent across study regions. Higher numbers of parasites have been linked to higher colony mortality [58], although this relationship might be non-linear [39]. Ravoet et al. 2013 [39] correlated the incidence of 18 parasite taxa and colony mortality and found that winter mortality steeply increased with the number of parasite species rising from 3 to 5, but that additional parasites had no further effect. In our study, the percentage of colonies with more than 5 detected parasite taxa was only 47 % in the group of feral colonies but 65 % in the group of managed colonies, hence the difference was likely within a relevant range.

Importantly, not only the number but also the composition of parasite taxa differed, meaning that it was not entirely random which parasites were less prevalent in feral colonies: Especially Trypanosomatidae, Chronic Bee Paralysis Virus and Deformed Wing Virus strains A and B were less frequently detected in feral colonies. These four parasites are all important since they can induce host mortality. Furthermore, they have been detected in other bee species, so they are also relevant in the context of honeybee-wild bee interactions [59,60]. The two trypanosomatid species *Chrithidia mellificae* and *Lotmaria passim* (among which we did not distinguish with our PCR assay) are unicellular parasites that colonize the bees' hindgut [61]. They have been experimentally shown to reduce the lifespan of individual workers [59] and their presence is associated with colony-level winter mortality [39]. Chronic bee paralysis virus (CBPV) causes the bee paralysis disease in adult workers, which involves symptoms like hair loss, undirected trembling walks, and loss of flight ability. The virus can spread in a colony via worker contact and eventually lead to its collapse [62]. Interestingly, CBPV seems to be an emerging threat, as indicated by a rapid rise of cases among Britain's apiaries during the last decade [63]. Given that in this study, its prevalence was 14.9% in managed colonies but only 1.6% in feral colonies, the latter are unlikely to represent an important dispersal route for the virus.

The reduction in the prevalence of Deformed Wing Viruses (DWV) A and B in feral compared to managed colonies is, ecologically, the most important detected difference since DWV are regarded a main cause of winter colony losses [64–66]. When transmitted during development, DWV directly kill their host at the pupal stage or cause body deformations of the emerging bee, leading to premature death [64]. Even infected bees that do not show overt symptoms have their life expectancy reduced by DWV, which can result in mass losses of bees on the colony level and its collapse during winter [66]. Importantly, the abundance of DWV in honeybee colonies and the severity of the resulting symptoms are tightly linked to

the co-occurrence with *V. destructor* [64,67]. While DWV were unproblematic before the invasion of *V. destructor*, the mite represented a new vector not only aiding the spread but also fuelling the evolution of the viruses, as the emergence and ongoing replacement of DWV-A by the presumably more virulent DWV-B indicates [67,68]. In turn, the mite's reproduction is enhanced by the presence of DWVs, making the mite-virus pair a deadly symbiosis [69]. Unfortunately, it was not possible in our study to investigate the levels of *V. destructor* infestation, because the numbers of mites on foragers are generally too low to make a reasonable analysis based on a few dozens of bees captured at the colony entrance. It would have required sampling pupae or young bees from the brood nest or assessing the rate at which dead mites naturally fall to the bottom of the nest cavity [70], but this was not possible in the case of the feral colonies. However, since DWVs and *Varroa* correlate [71,72], the lower prevalence of DWVs in feral colonies suggests that mite infestation levels were also reduced.

Our findings are in seeming conflict with a previous study from England which concluded that parasite pressure on feral colonies is relatively high [29]. Thompson et al. (2014) tested for 10 parasites and found that these were equally prevalent among managed and feral colonies, but the colony-level parasite abundance of one parasite, Deformed Wing Virus, was significantly higher in feral colonies (no difference was made between DWV-A and DWV-B). However, the sample of feral colonies analysed in their study was not random but only included colonies aged at least one year. We also found that the parasite prevalence in feral colonies aged at least one year (overwintered feral colonies) did not differ significantly from managed colonies and that they had relatively high loads of DWV-B (although not of DWV-A; Figure 5c). Therefore, had we only considered old feral colonies, we might have come to similar conclusions. However, we would have then seriously overestimated the parasite burden in the feral population because only about 10% of the feral colonies present in summer

are older than one year [28], and especially the young, newly founded feral colonies had their parasite burden reduced compared to managed colonies. These considerations demonstrate that it is important to know the population ecology of the feral honeybees under consideration when asking questions about their ecological impact, e.g., their contribution to the spread of bee diseases.

The feral honeybees investigated in this study are known to be recent descendants from colonies managed in apiaries [28], and therefore, their reduced parasite burden can only be explained by ecological/environmental, not genetic, differences from managed honeybees. By considering five colony types representing differences in colony age and management history (Table 1), we gained some insights into why parasite burden is reduced in feral colonies. We found that newly founded feral colonies had a lower parasite burden than both overwintered feral colonies and overwintered managed colonies (from which many of the young feral colonies directly descend). This supports the hypothesis that the natural process of reproductive swarming – the abandonment of the old nest, the pause of brood production, and the construction of fresh comb at a new dwelling place – leads to a temporal release from parasite pressure [9,10,12]. The high proportion of young, swarm-founded colonies in the feral population is one of the reasons for the overall significant difference in parasite burden between feral and managed colonies. In the managed population, the proportion of young colonies is much lower (about 50 %), and most young colonies are so-called nucleus colonies (in our sample: 75%), created by transferring several combs with brood from the mother colony into a new hive – a completely different founding mechanism. Since nucleus colonies directly inherit the parasites residing in the old combs and the brood, their parasite communities should rather resemble established (old) colonies. Indeed, parasite counts did neither differ significantly between nucleus colonies and overwintered managed colonies, nor between nucleus colonies and overwintered feral colonies, albeit overwintered managed

colonies had the highest numbers and a different community composition of parasites. This might be explained by the fact that beekeepers usually manage established colonies in such a way that brood is continuously produced, while nucleus colonies and overwintered feral colonies typically experience a brood pause in spring and thus a temporal reduction of breeding ground for parasites (Table 1) [9]. Importantly, young managed colonies founded by swarms ("hived swarms") were infested by a significantly higher number of microparasite taxa than young feral colonies. This showed that not only differences in population age structure, but also in the environment play a role in reducing parasite burden.

The most obvious environmental difference that has been demonstrated to affect parasite pressure is that managed hives are typically clustered in apiaries, while feral colonies are dispersed [14,15,31]. The crowding of colonies promotes random drifting and robbing behaviour, and both drifters and robbers can transfer parasites between hives [14,16,31]. Another highly consistent difference is that beekeepers conventionally keep their hives at ground level, while feral colonies typically nest in cavities several meters above the ground. The bees' preference for aerial cavities is thought to serve as a protection against predators, but a positive side effect might be that colonies dispose of sick bees more easily. For example, both CBPV and DWV cause the loss of flight ability, and flightless bees that fall out of the nest might struggle to find their way back when the entrance is high above the ground. There are more differences which might directly or indirectly affect the colonies' likelihood of becoming infected by parasites and of developing disease symptoms, e.g., the size of the nest entrance, the presence of landing boards to aid bees entering the hive, the insulation of the hive, the microclimate at the nest site, the frequency of disturbance, or the type of food consumed. Given the role parasites play in limiting survival of managed colonies in apiculture, it seems worth experimentally investigating these factors in more detail. For example, an interesting new question raised by our study is why swarms hived at an apiary

develop higher parasite loads than feral swarms, and how it is possible to restore the natural parasite-reducing effect of swarm-founding under apicultural management.

Table 1: Nonexclusive list of factors that might affect parasite burden, and their typical manifestation in different types of managed and feral honeybee colonies.

Factor	Managed colonies			Feral colonies		Assumed effect on parasite prevalence
	Overwintered colonies	Nucleus colonies	Hived swarms	Overwintered colonies	Founder colonies	
Time since acaricide treatment	<1 year	<1 year	<1 year	>1 year	>1 year / <1 year*	Treatment reduces pressure from mites and associated viruses
Pause of brood production in spring	no	yes / no[§]	yes	yes	yes	Brood pause cuts off reproduction of brood parasites
Presence of old comb in nest	yes	yes	no	yes	yes / no[#]	Old comb can be a source of parasites
Spatial arrangement of colonies	clustered			dispersed		Parasite transmission rates are increased when colonies are closer to each other
Height of nest entrance	low			high		Colonies might get rid of sick, flightless bees more quickly when nest entrance is high above the ground

* Newly founded feral colonies that directly descend from managed hives were usually treated against mites < 1 year before founding.

[§] Beekeepers can either produce a nucleus colony by taking combs with worker bees and brood from an established colony and let the new colony raise a queen from existing female larvae, in which case they create a brood pause, or directly introduce a mature queen to the new colony, in which case the nucleus colony does not experience a significant brood pause.

[#] Swarms prefer to occupy cavities that have been used by bees before, so they sometimes move into cavities still containing old comb.

Wild or feral honeybee colonies carrying many parasites and/or high parasite abundances would be a nuisance to apicultural disease management. However, we found that feral colonies have on average fewer parasites and lower parasite loads than managed colonies. This is partly explained by the effect of natural swarm reproduction and dispersal – a mechanisms of parasite reduction that might also be effective in other swarm-founding social

insects [73]. We conclude that it is unlikely that feral honeybees significantly contribute to the spread of bee parasites. On the contrary, new disease agents are probably primarily propagated by managed colonies, as indicated by the higher prevalence of two emerging viruses, CBPV and DWV-B, in the sample of managed hives. The management implication of this work is that the prevention of epidemics is no suitable argument for the often-practised removal or destruction of feral honeybee nests [74]. In fact, our data suggest that there is no conflict between the promotion of wild or feral honeybee populations and the management of bee diseases in apiculture. What remains unclear is how the various environmental differences between wild nests and hives at apiaries contribute to the reduced parasite burden in feral colonies. Some known natural parasite-reducing factors, e.g., the spatial separation of colonies and the periodic interruption of brood production, can readily be adopted by beekeepers to increase the health of managed honeybees [9,75,76].

Authors' contributions

P.L.K.: conceptualization, formal analysis, funding acquisition, investigation, methodology, visualization, writing–original draft, writing–review & editing; P.D..: formal analysis, investigation, methodology, validation, writing–review & editing; B.R.: conceptualization, investigation, methodology, writing–review & editing; S.R.: conceptualization, investigation, methodology, writing–review & editing; F.R.: conceptualization, investigation, methodology, writing–review & editing; I.S.D.: conceptualization, supervision, writing–review & editing; M.H.: resources, supervision, writing–review & editing.

Acknowledgements

We thank Luis Sikora, Norbert Wimmer and Kurt Zeimentz for sharing locations of woodpecker cavities which serve as nest sites for feral honeybees in German forests. We also

thank all beekeepers who granted us permission to sample bees from their hives. This study was supported German Federal Environmental Foundation (DBU).

References

1. Schmid-Hempel P. 1998 *Parasites in social insects*. Princeton University Press.

2. Bailey L. 1958 Wild Honeybees and Disease. *Bee World* **39**, 92–95. (doi:10.1080/0005772X.1958.11095044)

3. Ratnieks FW, Nowakowski J. 1989 Honeybee swarms accept bait hives contaminated with American foulbrood disease. *Ecol. Entomol.* **14**, 475–478. (doi:10.1111/j.1365-2311.1989.tb00951.x)

4. Fries I, Lindström A, Korpela S. 2006 Vertical transmission of American foulbrood (Paenibacillus larvae) in honey bees (Apis mellifera). *Vet. Microbiol.* **114**, 269–274. (doi:10.1016/j.vetmic.2005.11.068)

5. Fries I, Camazine S. 2001 Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. *Apidologie* **32**, 199–214. (doi:10.1051/apido:2001122)

6. Cremer S, Armitage SAO, Schmid-Hempel P. 2007 Social Immunity. *Curr. Biol.* **17**, 693–702. (doi:10.1016/j.cub.2007.06.008)

7. Arathi HS, Burns I, Spivak M. 2000 Ethology of hygienic behaviour in the honey bee Apis mellifera L. (Hymenoptera: Apidae): Behavioural repertoire of hygienic bees. *Ethology* **106**, 365–379. (doi:10.1046/j.1439-0310.2000.00556.x)

8. Simone M, Evans JD, Spivak M. 2009 Resin collection and social immunity in honey bees. *Evolution (N. Y).* **63**, 3016–3022. (doi:10.1111/j.1558-5646.2009.00772.x)

9. Loftus JC, Smith ML, Seeley TD. 2016 How Honey Bee Colonies Survive in the Wild :

Testing the Importance of Small Nests and Frequent Swarming. , 1–11. (doi:10.1371/journal.pone.0150362)

10. DeGrandi-Hoffman G, Ahumada F, Graham H. 2017 Are dispersal mechanisms changing the host-parasite relationship and increasing the virulence of varroa destructor (mesostigmata: Varroidae) in managed honey bee (hymenoptera: Apidae) colonies? *Environ. Entomol.* **46**, 737–746. (doi:10.1093/ee/nvx077)

11. Seeley TD. 2010 *Honeybee Democracy*. Princeton University Press.

12. Royce LA, Rossignol PA, Burgett DM, Stringer BA. 1991 Reduction of tracheal mite parasitism of honey bees by swarming. *Philos. Trans. - R. Soc. London, B* **331**, 123–129. (doi:10.1098/rstb.1991.0003)

13. Brosi BJ, Delaplane KS, Boots M, De Roode JC. 2017 Ecological and evolutionary approaches to managing honeybee disease. *Nat. Ecol. Evol.* **1**, 1250–1262. (doi:10.1038/s41559-017-0246-z)

14. Seeley TD, Smith ML. 2015 Crowding honeybee colonies in apiaries can increase their vulnerability to the deadly ectoparasite Varroa destructor. *Apidologie* **46**, 716–727. (doi:10.1007/s13592-015-0361-2)

15. Nolan MP, Delaplane KS. 2017 Distance between honey bee Apis mellifera colonies regulates populations of Varroa destructor at a landscape scale. *Apidologie* **48**, 8–16. (doi:10.1007/s13592-016-0443-9)

16. Peck DT, Seeley TD. 2019 Mite bombs or robber lures ? The roles of drifting and robbing in Varroa destructor transmission from collapsing honey bee colonies to their neighbors. *PLoS One* **14**, e0218392.

17. Martínez-López V, Ruiz C, De la Rúa P. 2022 Migratory beekeeping and its influence

on the prevalence and dispersal of pathogens to managed and wild bees. *Int. J. Parasitol. Parasites Wildl.* **18**, 184–193. (doi:10.1016/j.ijppaw.2022.05.004)

18. Goulson D, Hughes WOH. 2015 Mitigating the anthropogenic spread of bee parasites to protect wild pollinators. *Biol. Conserv.* **191**, 10–19. (doi:10.1016/j.biocon.2015.06.023)

19. Traynor KS, Mondet F, de Miranda JR, Techer M, Kowallik V, Oddie MAY, Chantawannakul P, McAfee A. 2020 Varroa destructor: A Complex Parasite, Crippling Honey Bees Worldwide. *Trends Parasitol.* **36**, 592–606. (doi:10.1016/j.pt.2020.04.004)

20. Wilfert L, Long G, Leggett HC, Schmid-Hempel P, Butlin R, Martin SJM, Boots M. 2016 Honeybee disease: Deformed wing virus is a recent global epidemic in honeybees driven by Varroa mites. *Science (80-.).* **351**, 594–597. (doi:10.1126/science.aac9976)

21. Bartlett LJ. 2022 Frontiers in effective control of problem parasites in beekeeping. *Int. J. Parasitol. Parasites Wildl.* **17**, 263–272. (doi:10.1016/j.ijppaw.2022.03.003)

22. Loper GM, Sammataro D, Finley J, Cole J. 2006 Feral honey bees in Southern Arizona, 10 years after Varroa infestation. *Am. Bee J.* **146**, 521–524.

23. Kraus B, Page RER. 1995 Effect of Varroa jacobsoni (Mesostigmata: Varroidae) on feral Apis mellifera (Hymenoptera: Apidae) in California. *Environ. Entomol.* **24**, 1473–1480. (doi:10.1093/ee/24.6.1473)

24. Mikheyev AS, Tin MMY, Arora J, Seeley T. 2016 Museum samples reveal rapid evolution by wild honey bees exposed to a novel parasite. *Nat. Commun.* **6**, 1–8. (doi:10.1038/ncomms8991)

25. Bozek K, Rangel J, Arora J, Tin MM., Crotteau E, Loper GM, Fewell J, Mikheyev AS.

2018 Parallel genomic evolution of parasite tolerance in wild honey bee populations. , 29–30.

26. Villa JD, Bustamante DM, Dunkley JP, Escobar LA. 2008 Changes in honey bee (Hymenoptera: Apidae) colony swarming and survival pre- and postarrival of Varroa destructor (Mesostigmata: Varroidae) in Louisiana. *Ann. Entomol. Soc. Am.* **101**, 867–871.)

27. Thompson CE. 2012 The health and status of the feral honeybee (Apis mellifera sp) and Apis mellifera mellifera population of the UK. The Unversity of Leeds.

28. Kohl PL, Rutschmann B, Steffan-Dewenter I. 2022 Population demography of feral honeybee colonies in Central European forests. *R. Soc. Open Sci.* (doi:10.1098/rsos.220565) (*in print*).

29. Thompson CE, Biesmeijer JC, Allnutt TR, Pietravalle S, Budge GE. 2014 Parasite pressures on feral honey bees (Apis mellifera sp.). *PLoS One* **9**, e105164. (doi:10.1371/journal.pone.0105164)

30. Manning R, Lancaster K, Rutkay A, Eaton L. 2007 Survey of feral honey bee (Apis mellifera) colonies for Nosema apis in Western Australia. *Aust. J. Exp. Agric.* **47**, 883–886.

31. Lindström A, Korpela S, Fries I. 2008 Horizontal transmission of Paenibacillus larvae spores between honey bee (Apis mellifera) colonies through robbing. *Apidologie* **39**, 515–522. (doi:10.1051/apido:2008032)

32. Frey E, Rosenkranz P. 2014 Autumn invasion rates of Varroa destructor (Mesostigmata: Varroidae) into honey bee (Hymenoptera: Apidae) colonies and the resulting increase in mite populations. *J. Econ. Entomol.* **107**, 508–515.

33. Piot N *et al.* 2022 Honey bees and climate explain viral prevalence in wild bee communities on a continental scale. *Sci. Rep.* **12**, 1–12. (doi:10.1038/s41598-022-05603-2)

34. Fürst M a., McMahon DP, Osborne JL, Paxton RJ, Brown MJF. 2014 Disease associations between honeybees and bumblebees as a threat to wild pollinators. *Nature* **506**, 364–366. (doi:10.1038/nature12977)

35. Tehel A, Streicher T, Tragust S, Paxton RJ. 2022 Experimental cross species transmission of a major viral pathogen in bees is predominantly from honeybees to bumblebees. *Proc. R. Soc. B Biol. Sci.* **289**. (doi:10.1098/rspb.2021.2255)

36. D'Alvise P, Seeburger V, Gihring K, Kieboom M, Hasselmann M. 2019 Seasonal dynamics and co - occurrence patterns of honey bee pathogens revealed by high - throughput RT - qPCR analysis. *Ecol. Evol.* , 1–12. (doi:10.1002/ece3.5544)

37. 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**, 1–16. (doi:10.1371/journal.pone.0142031)

38. Garbuzov M, Balfour NJ, Shackleton K, Al Toufailia H, Scandian L, Ratnieks FLW. 2020 Multiple methods of assessing nectar foraging conditions indicate peak foraging difficulty in late season. *Insect Conserv. Divers.* **13**, 532–542. (doi:10.1111/icad.12420)

39. Ravoet J, Maharramov J, Meeus I, De Smet L, Wenseleers T, Smagghe G, de Graaf DC. 2013 Comprehensive Bee Pathogen Screening in Belgium Reveals Crithidia mellificae as a New Contributory Factor to Winter Mortality. *PLoS One* **8**. (doi:10.1371/journal.pone.0072443)

40. Weigand M, Staab J, Wurm M, Taubenböck H. 2020 Spatial and semantic effects of

LUCAS samples on fully automated land use/land cover classification in high-resolution Sentinel-2 data. *Int. J. Appl. Earth Obs. Geoinf.* **88**, 102065. (doi:10.1016/j.jag.2020.102065)

41. Budge GE *et al.* 2010 The occurrence of Melissococcus plutonius in healthy colonies of Apis mellifera and the efficacy of European foulbrood control measures. *J. Invertebr. Pathol.* **105**, 164–170. (doi:10.1016/j.jip.2010.06.004)

42. Cepero A, Martín-Hernández R, Prieto L, Gómez-Moracho T, Martínez-Salvador A, Bartolomé C, Maside X, Meana A, Higes M. 2015 Is Acarapis woodi a single species? A new PCR protocol to evaluate its prevalence. *Parasitol. Res.* **114**, 651–658. (doi:10.1007/s00436-014-4229-6)

43. Evans JD. 2006 Beepath: An ordered quantitative-PCR array for exploring honey bee immunity and disease. *J. Invertebr. Pathol.* **93**, 135–139. (doi:10.1016/j.jip.2006.04.004)

44. Locke B, Forsgren E, Fries I, de Miranda JR. 2012 Acaricide treatment affects viral dynamics in Varroa destructor-infested honey bee colonies via both host physiology and mite control. *Appl. Environ. Microbiol.* **78**, 227–235. (doi:10.1128/AEM.06094-11)

45. Lourenço AP, Mackert A, Cristino ADS, Simões ZLP. 2008 Validation of reference genes for gene expression studies in the honey bee, Apis mellifera, by quantitative real-time RT-PCR. *Apidologie* **39**, 372–385. (doi:10.1051/apido:2008015)

46. Martínez J, Simon V, Gonzalez B, Conget P. 2010 A real-time PCR-based strategy for the detection of Paenibacillus larvae vegetative cells and spores to improve the diagnosis and the screening of American foulbrood. *Lett. Appl. Microbiol.* **50**, 603–610. (doi:10.1111/j.1472-765X.2010.02840.x)

47. Papp T, Spann D, Marschang RE. 2014 Development and use of a real-time polymerase chain reaction for the detection of group II invertebrate iridoviruses in pet lizards and prey insects. J. Zoo Wildl. Med., 45(2), 219–27./stable/24551086. *J. Zoo Wildl. Med.* **45**, 219–227.

48. R Core Team. 2022 R: A Language and Environment for Statistical Computing. *R Found. Stat. Comput.* See https://www.r-project.org/.

49. Wickham H. 2016 *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

50. Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Mächler M, Bolker BM. 2017 glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* **9**, 378–400. (doi:10.32614/rj-2017-066)

51. Brooks ME, Kristensen K, Darrigo MR, Rubim P, Uriarte M, Bruna E, Bolker BM. 2019 Statistical modeling of patterns in annual reproductive rates. *Ecology* **100**, 1–7. (doi:10.1002/ecy.2706)

52. Lenth R V. 2022 emmeans: Estimated Marginal Means, aka Least-Squares Means.

53. Hothorn T, Bretz F, Westfall P. 2008 Simultaneous Inference in General Parametric Models. *Biometrical J.* **50**, 346–363.

54. Oksanen J *et al.* 2022 vegan: Community Ecology Package.

55. Borcard D, Legendre P. 2002 All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices Daniel. *Ecol. Modell.* **153**, 51–68.

56. Blaker H. 2000 Confidence curves and improved exact confidence intervals for discrete distributions. *Can. J. Stat.* **28**, 783–798.

57. Stevenson M *et al.* 2022 epiR: Tools for the Analysis of Epidemiological Data.

58. vanEngelsdorp D *et al.* 2009 Colony collapse disorder: A descriptive study. *PLoS One* **4**. (doi:10.1371/journal.pone.0006481)

59. Strobl V, Yañez O, Straub L, Albrecht M, Neumann P. 2019 Trypanosomatid parasites infecting managed honeybees and wild solitary bees. *Int. J. Parasitol.* **49**, 605–613. (doi:10.1016/j.ijpara.2019.03.006)

60. Tehel A, Brown MJF, Paxton RJ. 2016 ScienceDirect Impact of managed honey bee viruses on wild bees. *Curr. Opin. Virol.* **19**, 16–22. (doi:10.1016/j.coviro.2016.06.006)

61. Schwarz RS, Bauchan GR, Murphy CA, Ravoet J, De Graaf DC, Evans JD. 2015 Characterization of two species of trypanosomatidae from the Honey Bee Apis mellifera: Crithidia mellificae Langridge and McGhee, and Lotmaria passim n. gen., n. sp. *J. Eukaryot. Microbiol.* **62**, 567–583. (doi:10.1111/jeu.12209)

62. Ribière M, Olivier V, Blanchard P. 2010 Chronic bee paralysis: A disease and a virus like no other? *J. Invertebr. Pathol.* **103**, S120–S131. (doi:10.1016/j.jip.2009.06.013)

63. Budge GE, Simcock NK, Holder PJ, Shirley MDF, Brown MA, Van Weymers PSM, Evans DJ, Rushton SP. 2020 Chronic bee paralysis as a serious emerging threat to honey bees. *Nat. Commun.* **11**. (doi:10.1038/s41467-020-15919-0)

64. de Miranda JR, Genersch E. 2010 Deformed wing virus. *J. Invertebr. Pathol.* **103**, S48–S61. (doi:10.1016/j.jip.2009.06.012)

65. Genersch E *et al.* 2010 The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. *Apidologie* **41**, 332–352. (doi:10.1051/apido/2010014)

66. Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P. 2012 Dead or alive: Deformed

wing virus and varroa destructor reduce the life span of winter honeybees. *Appl. Environ. Microbiol.* **78**, 981–987. (doi:10.1128/AEM.06537-11)

67. Paxton RJ *et al.* 2022 Epidemiology of a major honey bee pathogen, deformed wing virus: potential worldwide replacement of genotype A by genotype B. *Int. J. Parasitol. Parasites Wildl.* **18**, 157–171. (doi:10.1016/j.ijppaw.2022.04.013)

68. McMahon DP, Natsopoulou ME, Doublet V, Fürst M, Weging S, Brown MJF, Gogol-Döring A, Paxton RJ. 2016 Elevated virulence of an emerging viral genotype as a driver of honeybee loss. *Proc. R. Soc. B Biol. Sci.* **283**. (doi:10.1098/rspb.2016.0811)

69. Di G, Annoscia D, Margiotta M, Ferrara R, Varricchio P, Zanni V. 2016 A mutualistic symbiosis between a parasitic mite and a pathogenic virus undermines honey bee immunity and health. *Proc. Natl. Acad. Sci. U. S. A.* **113**, 3203–3208. (doi:10.1073/pnas.1523515113)

70. Dietemann V *et al.* 2013 Standard methods for varroa research. *J. Apic. Res.* **52**, 1–54. (doi:10.3896/IBRA.1.52.1.09)

71. Norton AM, Remnant EJ, Tom J, Buchmann G, Blacquiere T, Beekman M. 2021 Adaptation to vector-based transmission in a honeybee virus. *J. Anim. Ecol.* **90**, 2254–2267. (doi:10.1111/1365-2656.13493)

72. Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P. 2012 Predictive markers of honey bee colony collapse. *PLoS One* **7**. (doi:10.1371/journal.pone.0032151)

73. McGlynn TP. 2012 The ecology of nest movement in social insects. *Annu. Rev. Entomol.* **57**, 291–308. (doi:10.1146/annurev-ento-120710-100708)

74. Taylor MA, Goodwin RM, McBrydie HM, Cox HM. 2007 Destroying managed and feral honey bee (Apis mellifera) colonies to eradicate honey bee pests. *New Zeal. J.*

Crop Hortic. Sci. **35**, 313–323. (doi:10.1080/01140670709510197)

75. Dynes TL, Berry JA, Delaplane KS, Brosi BJ, De Roode JC. 2019 Reduced density and visually complex apiaries reduce parasite load and promote honey production and overwintering survival in honey bees. *PLoS One* **14**, 1–16. (doi:10.1371/journal.pone.0216286)

76. Büchler R *et al.* 2020 Summer brood interruption as integrated management strategy for effective Varroa control in Europe. *J. Apic. Res.* **59**, 764–773. (doi:10.1080/00218839.2020.1793278)