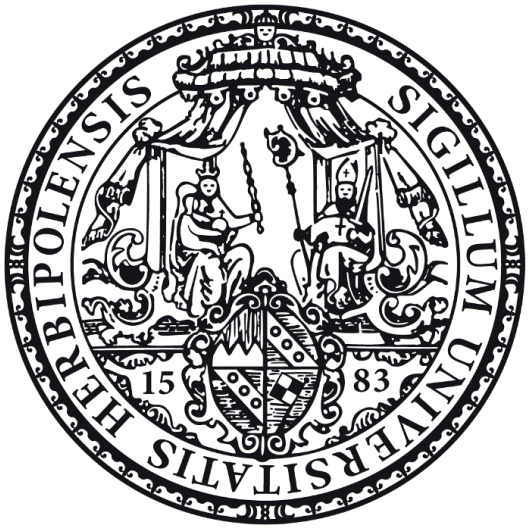
The buzz beyond the beehive: population demography, parasite burden and limiting factors of wild-living honeybee colonies in Germany

Das Summen fern des Bienenstocks: Populationsdemographie, Parasitenlast und limitierende Faktoren wildlebender Honigbienenvölker in Deutschland



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„*Die Zahl der im Walde lebenden ‚wilden' Bienenvölker ist in Franken heute nur noch gering. Unsere neuzeitliche Forstwirtschaft mit ihrer geregelten Umtriebszeit und systematischen Entfernung hohler Bäume hat die Waldbiene ihrer wichtigsten Nistgelegenheit beraubt, so daß sie wie auch andere Arten unserer Tierwelt mehr und mehr verschwinden mußte.*"

"The number of forest-dwelling wild honeybee colonies in Franconia is low today. Our modern forestry, with its fixed rotation periods and systematic removal of hollow trees, has bereaved the forest bee of her most important nesting site, so that she, like other animal species, gradually disappeared."

F. K. Stoeckhert (1933)

„*Die Zahl der im Walde lebenden Bienenvölker scheint doch größer zu sein, als man gewöhnlich annimmt. Sie entziehen sich nur allzu leicht der Beobachtung; denn die Fluglöcher liegen vielfach in der Laubkrone versteckt sehr hoch über dem Erdboden*."

"The number of forest-dwelling honeybee colonies seems to be larger than is commonly assumed. They all too easily evade our notice because flight entrances are typically well-hidden by the tree canopy, high above the ground."

F. K. Stoeckhert (1954)

Dedicated to all honeybee swarms that take the risk of leaving their hives

A honeybee swarm shortly after having founded a nest in a cavity made by the black woodpecker in a beech tree (Photo credit: Dimi Dumortier)

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Summary

The western honeybee (*Apis mellifera*) is widely known as the honey producer and pollinator managed by beekeepers but neglected as a wild bee species. Central European honeybee populations have been anthropogenically disturbed since about 1850 through introgression and moderate artificial selection but have never been truly domesticated due to a lack of mating control. While their decline in the wild was historically attributed to the scarcity of nesting cavities, a contemporary view considers the invasion of the parasitic mite *Varroa destructor* in the 1970s as the major driver. However, there are no longitudinal population data available that could substantiate either claim. Based on the insight that introduced European honeybees form viable wild populations in eastern North America and reports on the occurrence of wild-living colonies from various European countries, we systematically studied the ecology of wild-living honeybees in Germany. First, we investigated whether wild-living honeybees colonising German forests form a self-sustaining population. Second, we asked how the parasite burden of wild-living colonies relates to that of managed colonies. And third, we explored whether the winter mortality of wild-living colonies is associated with parasite burden, nest depredation, or the lack of resources on the landscape scale.

Between 2017 and 2021, we monitored listed trees with black woodpecker cavities for honeybees in the managed forests of three study regions (Swabian Alb, counties Coburg and Lichtenfels, county Weilheim-Schongau). Continuity of occupation was determined using microsatellite genetic markers. Wild-living colonies predictably colonised forests in summer, when about 10% of all cavities were occupied. The annual colony survival rate and colony lifespan (based on N=112 colonies) were 10.6% and 0.6 years, with 90% of colonies surviving summer (July–September), 16% surviving winter (September–April), and 72% surviving spring (April–July). The average maximum and minimum colony densities were 0.23 (July) and 0.02 (April) colonies per km^2. During the (re-)colonisation of forests in spring, swarms preferred cavities that had already been occupied by other honeybee colonies. We estimate the net reproductive rate of the population to be R_0= 0.318, meaning that it is currently not self-sustaining but maintained by the annual immigration of swarms from managed hives. The wild-living colonies are feral in a behavioural sense.

We compared the occurrence of 18 microparasites among feral colonies (N=64) and managed colonies (N=74) using qPCR. Samples were collected in four regions (the three regions mentioned above and the city of Munich) in July 2020; they consisted of 20 workers per colony captured at flight entrances. We distinguished five colony types representing differences in colony age and management histories. Besides strong regional variation, feral colonies consistently hosted fewer microparasite taxa (median: 5, range 1–8) than managed colonies (median: 6, range 4–9) and had

different parasite communities. Microparasites that were notably less prevalent among feral colonies were Trypanosomatidae, Chronic bee paralysis virus, and Deformed wing viruses A and B. In the comparison of five colony types, 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. This suggests that the natural mode of colony reproduction by swarming, which creates pauses in brood production, and well-dispersed nests, which reduce horizontal transmission, explain the reduced parasite burden in feral compared to managed colonies.

To explore the roles of three potential drivers of feral colony winter mortality, we combined colony observations gathered during the monitoring study with data on colony-level parasite burden, observations and experiments on nest depredation, and landscape analyses. There was no evidence for an effect of summertime parasite burden on subsequent winter mortality: colonies that died (N=57) did not have a higher parasite burden than colonies that survived (N=10). Camera traps (N=15) installed on cavity trees revealed that honeybee nests are visited by a range of vertebrate species throughout the winter at rates of up to 10 visits per week. Four woodpecker species, great tits, and pine martens acted as true nest depredators. The winter survival rate of colonies whose nest entrances were protected by screens of wire mesh (N=32) was 50% higher than that of colonies with unmanipulated entrances (N=40). Analyses of land cover maps revealed that the landscapes surrounding surviving colonies (N=19) contained on average 6.4 percentage points more resource-rich cropland than landscapes surrounding dying colonies (N=94).

We estimate that tens of thousands of swarms escape from apiaries each year to occupy black woodpecker cavities and other hollow spaces in Germany and that feral colonies make up about 5% of the regional honeybee populations. They are unlikely to contribute disproportionately to the spread of bee diseases. Instead, by spatially complementing managed colonies, they contribute to the pollination of wild plants in forests. Honeybees occupying tree cavities likely have various effects on forest communities by acting as nest site competitors or prey, and by accumulating biomass in tree holes. Nest depredation (a consequence of a lack of well-protected nest sites) and food resource limitation seem to be more important than parasites in hampering feral colony survival. The outstanding question is how environmental and intrinsic factors interact in preventing population establishment. Nest boxes with movable frames could be used to better study the environmental drivers of feral colonies' mortality. Pairs of wild (self-sustaining) and managed populations known to exist outside Europe could provide answers to whether modern apiculture creates honeybee populations maladapted to life in the wild. In Europe, large continuous forests might represent evolutionary refuges for wild honeybees.

Zusammenfassung

Die Honigbiene (*Apis mellifera*) ist als Nutztier weitbekannt, doch als Wildtier vernachlässigt. Seit etwa 1850 sind ihre Populationen in Mitteleuropa durch Introgression und moderate künstliche Selektion vom Menschen beeinflusst. Die Art wurde jedoch aufgrund fehlender Paarungskontolle nie wirklich domestiziert. Früher wurde der Rückgang wildlebender Honigbienen dem Verlust geeigneter Nistplätze zugeschrieben. Heute wird meist die Bienenmilbe *Varroa destructor* als Hauptursache angenommen. Es gibt allerdings keine Langzeitdaten, welche diese Annahmen stützen könnten. Basierend auf der Erkenntnis, dass eingeführte Honigbienen in Nordamerika stabile wilde Populationen bilden, und aufgrund von Berichten über das Vorkommen wildlebender Bienenvölker in verschiedenen Ländern Europas, widmeten wir uns dem systematischen Studium wildlebender Honigbienen in Deutschland. Zunächst untersuchten wir, ob waldbewohnende Bienenvölker eine selbsterhaltende Population bilden. Zweitens stellten wir die Frage, inwiefern sich wildlebende und imkerlich gehaltene Völker in ihrer Parasitenlast unterscheiden. Drittens testeten wir, ob Winterverluste wildlebender Bienenvölker mit Parasitendruck, Nestprädation oder mangelndem Nahrungsangebot auf Landschaftsebene in Verbindung stehen.

In Wirtschaftswäldern dreier Untersuchungsgebiete (Schwäbische Alb, Landkreise Coburg und Lichtenfels, Landkreis Weilheim-Schongau) kontrollierten wir zwischen 2017 und 2021 bekannte Höhlenbäume des Schwarzspechts auf Besiedlung durch Honigbienen. Das Überleben einzelner Bienenvölker wurde zusätzlich mittels Analyse von Mikrosatelliten DNA überprüft. Nach verlässlichem Muster besiedelten Honigbienen jeden Sommer etwa 10% der Baumhöhlen. Die jährliche Überlebensrate und die Lebenserwartung der Völker (N=112) betrugen 10,6% und 0,6 Jahre, wobei 90% den Sommer (Juli–September), 16% den Winter (September–April) und 72% das Frühjahr (April–Juli) überlebten. Die durchschnittliche maximale (Juli) und minimale (April) Koloniedichte betrug 0,23 bzw. 0,02 Bienenvölker pro km^2. Während der (Wieder)Besiedlung von Wäldern im Frühjahr bevorzugten Bienenschwärme solche Baumhöhlen, welche zuvor schon von Bienen besiedelt worden waren. Die Nettoreproduktionsrate der wildlebenden Population wird auf R_0= 0,318 geschätzt, was bedeutet, dass diese zurzeit nicht selbsterhaltend ist, sondern durch die jährliche Einwanderung von Bienenschwärmen aus der Imkerei aufrechterhalten wird.

Wir untersuchten wildlebende (N=64) und imkerlich gehaltene Bienenvölker (N=74) auf den Befall mit 18 verschiedenen Mikroparasiten mittels qPCR. Die Proben stammten aus den drei oben genannten Gebieten sowie aus dem Stadtgebiet von München. Eine Probe bestand aus 20 Arbeiterinnen, welche am Flugloch gefangen wurden. Wir unterschieden fünf Kolonietypen aufgrund des Alters (jünger oder älter als ein Jahr) und der unmittelbaren Geschichte der Bewirtschaftung durch Imkerinnen und Imker. Abgesehen von regionalen Unterschieden in der Parasitenlast waren wildlebende Völker mit einer geringeren Anzahl Parasitentaxa befallen (Median: 5, Spanne: 1–8)

als imkerlich gehaltene Völker (Median: 6, Spanne: 4–9) und wiesen eine veränderte Zusammensetzung von Parasiten auf. Seltener bei wildlebenden Bienenvölkern waren besonders Trypanosomatidae, das Chronische-Paralysevirus, sowie die Flügeldeformationsviren A und B. Im Vergleich der fünf Kolonietypen war die Parasitenlast bei neu gegründeten wildlebenden Völkern am geringsten, intermediär bei überwinterten wildlebenden Völkern und Brutablegern, und am höchsten bei überwinterten Wirtschaftsvölkern und bei durch Schwärme gegründeten imkerlich gehaltenen Völkern. Dies deutet darauf hin, dass das Schwärmen (Entstehung von Brutpausen) sowie die größere Distanz zwischen Nestern (Verminderung der horizontalen Krankheitsübertragung) die geringere Parasitenlast wildlebender Bienenvölker erklären.

Wir kombinierten Beobachtungen zum Winterüberleben aus dem Monitoring mit Daten zur Parasitenlast, mit Beobachtungen und Experimenten zur Nestprädation und mit Landschaftsanalysen. Es ergab sich kein Hinweis auf einen Zusammenhang zwischen Parasitenlast im Sommer und anschließendem Überwinterungserfolg: Völker, welche den Winter nicht überlebten (N=57), hatten zuvor keine höhere Parasitenlast als solche, welche den Winter überlebten (N=10). Kamerafallen (N=15) offenbarten, dass Honigbienennester im Winter von einer Vielzahl von Vögeln und Säugern mit bis zu 10 Besuchen pro Woche heimgesucht werden. Vier Spechtarten, Kohlmeisen und Baummarder wurden als echte Nestplünderer identifiziert. Bienenvölker, deren Nesteingang mit Maschendraht geschützt war (N=32), hatten eine 50% höhere Winterüberlebensrate als Völker ohne Schutz (N=40). Die Analyse von Landnutzungskarten zeigte, dass sich Bienenvölker, welche den Winter überlebten (N=19), in Landschaften mit durchschnittlich 6,4% höherem Anteil von Ackerflächen befanden als solche, die den Winter nicht überlebten (N=94).

Wir schätzen, dass in Deutschland jährlich zehntausende Schwärme von Bienenständen entfliehen, um sich in Spechthöhlen oder anderen Hohlräumen anzusiedeln. Der Anteil wildlebender Völker an der Gesamtbienenpopulation beträgt im Sommer etwa 5%. Sie spielen vermutlich eine untergeordnete Rolle bei der Verbreitung von Bienenkrankheiten. Durch die Ergänzung imkerlich gehaltener Völker in Waldgebieten tragen sie zur Bestäubung waldbewohnender Pflanzenarten bei. Die Besiedlung von Baumhöhlen sollte vielseitige Auswirkungen auf Lebensgemeinschaften im Wald haben: Bienenvölker konkurrieren um Nistplätze, sind reiche Beute im Winter und akkumulieren organisches Material. Nestprädation (eine Folge des Mangels an sicheren Nisthöhlen) und Ressourcenlimitierung spielen offenbar derzeit eine größere Rolle als Parasiten bei der Erklärung von Winterverlusten. Eine offene Frage ist, inwiefern Umwelt und genetische Dispositionen die Etablierung wilder Honigbienenpopulationen verhindern. Künstliche Nistkästen könnten genutzt werden, um die Rolle von Umweltfaktoren genauer zu untersuchen. Populationen wilder Honigbienen außerhalb Europas könnten Erkenntnisse dazu liefern, inwiefern sich die moderne Imkerei auf die Anpassungen der Honigbienen als Wildtier auswirkt. In Europa könnten große zusammenhängende Waldgebiete als evolutionäre Refugien für wilde Honigbienen dienen.

Acknowledgements

Like for the bees in the forest, the environment of doctoral students is crucial to their success. I therefore would like to first express my deepest gratitude to my primary supervisor Ingolf Steffan-Dewenter. When I came to Würzburg for my master studies in 2015, Ingolf integrated me into the Dept. of Animal Ecology right away. He was a great inspiration for broad ecological thinking and supported me in engaging in different research directions. By kindly hiring me as a "predoc" after I had obtained my master's degree, he allowed me to finish previous work and develop the research proposal that led to this thesis. Thank you Ingolf for your guidance and for your trust that following wild-living honeybee colonies could eventually lead to a dissertation!

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Chapter one
General introduction

Insect decline, the pollination crisis, and the neglect of the honeybee in the wild

In the face of the widespread population decline of flower-visiting insects driven by land-use change, agricultural intensification, environmental pollution and climate change, the ecological process of insect pollination is at risk (Kearns, Inouye & Waser, 1998; Potts et al., 2010a; Goulson et al., 2015; Wagner, 2020; Dicks et al., 2021). This not only affects plant communities in natural habitats (Biesmeijer et al., 2006); the decline of bees, wasps, flies, beetles, and butterflies is also problematic for agricultural production itself, given that the fruit set of many crops depends on insect pollination (Klein et al., 2007; Gallai et al., 2009; Leonhardt et al., 2013). The European strategy against the pollination deficit mainly focuses on promoting managed honeybees (*Apis mellifera*) (Williams, 2002; Council of Europe, 2004). This eusocial bee species is regarded as indispensable for pollination in large crop fields thanks to its natural tendency to exploit mass-flowering events, the high number of foragers per colony and the possibility to translocate hives according to agricultural demands (Morse, 1991; Rollin & Garibaldi, 2019). Honeybees are also generally considered beneficial for natural habitats because they visit the flowers of many wild plant species (Hung et al., 2018), and their large foraging ranges enable pollination over long distances (Dick, 2001; Ratnieks & Shackleton, 2015; Grüter & Hayes, 2022). However, it has been questioned whether modern apicultural practices are suited to preserve the diversity of honeybees in Europe (Requier et al., 2019a; Panziera et al., 2022). Furthermore, the delivery of pollination services benefits from the interactions and complementarity of a diverse range of – mostly wild – insect pollinators (Greenleaf & Kremen, 2006; Brittain, Kremen & Klein, 2013; Garibaldi et al., 2013; Mashilingi et al., 2022), and these do not benefit from apiculture (Iwasaki & Hogendoorn, 2022).

Beekeepers provide their bees with virtually unlimited access to high-quality nest sites (hives) and food (mass-flowering crops or sugar water), so the number of managed colonies mainly

depends on the number of people that keep bees, their training, and their operation sizes, which are all determined by socio-economic rather than environmental factors (Potts et al., 2010b; VanEngelsdorp & Meixner, 2010; Moritz & Erler, 2016). For example, even in a mid-latitude industrialised country such as Germany, where there are ongoing declines of wild insect populations associated with habitat loss (Seibold et al., 2019), the numbers of beekeepers and managed hives have risen during the last decade (Deutscher Imkerbund, 2020). Unfortunately, the growth of managed honeybee populations does not guarantee the conservation of even this single species. The bees propagated by apiculture are not always of local origin and often stem from breeding programmes selecting for traits that are beekeeper-friendly but do not increase fitness under natural conditions (Panziera et al., 2022).

Increasing the densities of managed honeybee colonies can also aggravate existing pressures on wild pollinators (Herrera, 2020). Due to their efficient social foraging strategy, honeybees can quickly deplete rewarding food patches and outcompete other flower visitors (Wignall et al., 2020). The density of honeybee colonies would not naturally exceed about one colony per km^2 in temperate climate regions (reviewed by Seeley, 2019). However, the density of managed colonies is typically much higher (about four colonies per km^2 on average, Chauzat et al., 2013) and, when managed hives are artificially aggregated in apiaries, the density of honeybee foragers is amplified further, exacerbating the problem of limited floral resources for wild pollinators (Elbgami et al., 2014; Lindström et al., 2016; Henry & Rodet, 2018). Furthermore, several apicultural practices, including (but not limited to) the transfer of bees and bee products between disparate regions or the placement of colonies next to each other, promote the spread of bee parasites (Goulson & Hughes, 2015; Seeley & Smith, 2015; Martínez-López, Ruiz & De la Rúa, 2022). Since many parasites of honeybees, especially viruses, can spill over to other insects through the shared use of flowers, modern beekeeping can also increase disease prevalence among wild pollinators (Fürst et al., 2014; Burnham et al., 2021; Piot et al., 2022; Tehel et al., 2022).

Given the mounting evidence for the negative effects of high honeybee densities on wild pollinator communities (Iwasaki & Hogendoorn, 2022), it has been repeatedly argued for banning honeybees from conservation areas (e.g., Geldmann & González-Varo, 2018). Unfortunately, this perspective entirely neglects the fact that *Apis mellifera* not only exists as a managed species but also as a wild one (Kohl & Rutschmann, 2018; Requier et al., 2019a; Seeley, 2019). Wild-living honeybees can be expected to face similar environmental challenges as other wild bees (Rutschmann et al., 2022), and they are the first to be affected by the pressures exerted by globalised apiculture (Pirk, Crewe & Moritz, 2017). Where the honeybee is native, conserving or re-establishing wild honeybee populations at natural (moderate) densities is arguably in the interest of both nature conservation and the agricultural sector. Stable wild populations of locally adapted honeybees would guarantee the sustainable provision of the species' ecosystem functions

and exert less pressure on other flower-visiting insects than apiculture. This thesis is dedicated to the investigation of wild-living honeybees in Germany and, therefore, will contribute to a better understanding and conservation of insect wildlife in the Anthropocene (Wagner, 2020).

A brief natural and cultural history of temperate-adapted western honeybees with a focus on central Europe

Biogeographic origins

The western honeybee separated an estimated six to eight million years ago from a common ancestor with the Southeast Asian cavity-nesting honeybees, probably somewhere in the Middle East or Central Asia (Ruttner, 1988a; Garnery, Cornuet & Solignac, 1992; Dogantzis et al., 2021). During its range expansions into Western Asia, Africa, and Europe, it diverged into several evolutionary lineages (Dogantzis et al., 2021). Europe was independently colonised by two lineages via two different geographic routes. Bees of the so-called "M lineage" entered Europe from the northeast and populated regions north of the Caspian and the Black Sea, north of the Carpathian Mountains, north and west of the Alps, and the Iberian Peninsula, whereas bees of the so-called "C lineage" colonised southeastern Europe and Italy via Asia Minor (Garnery, Cornuet & Solignac, 1992; Dogantzis et al., 2021). The European honeybee subspecies recognised today only differentiated between one million and 30,000 years ago, when glacial periods led to the geographic isolation of populations in Mediterranean refuges (Chen et al., 2016; Dogantzis et al., 2021). With the re-expansion of broadleaf forests after the last glacial period, honeybees re-colonised most of the continent except for high mountains and latitudes beyond 60 degrees north. Central Europe was naturally home to two subspecies, the European dark bee, *A. m. mellifera* (M lineage), which occurred north of the Alps and the Carpathians, and the Carnolian honeybee (*A. m. carnica*, C lineage), which is native to the southeastern foothills of the Alps and the Pannonian Basin (Ruttner, 1988a).

Life-history strategy

Honeybees feature several characteristics unique among the European bee fauna, which are explained by the (sub)tropical origin of the genus *Apis* (Ruttner, 1988a). While their closest European relatives, the bumblebees (genus *Bombus*), form small annual colonies, which are solitarily founded by hibernated queens each spring (Goulson, 2010), honeybees live in perennial colonies with long-lived queens and several thousand workers. Their mode of reproduction is colony fission, whereby a swarm of bees and a queen leave the colony and found a new nest at a different site (Winston, 1991). A common feature of all *Apis* bees is that when forage is abundant, they collect a surplus of nectar, which they concentrate into honey and store in beeswax combs. The ability to accumulate high-caloric food reserves probably evolved in subtropical forest

ecosystems to overcome shorter periods of drought or heavy rain but was a prerequisite for the colonisation of regions with long winters (Seeley, 1985; Ruttner, 1988a). African *A. mellifera* can track changes in food availability by making seasonal colony migrations, and they have little constraints on colony size, so they produce multiple small offspring colonies in multiple swarming bouts per year (Winston, Taylor & Otis, 1983). Honeybees living in temperate climate regions, in turn, must bridge about six months of adverse weather without foraging. To successfully hibernate, they need around 15 kg of stored honey and a large worker population of about 5,000 bees in autumn (Seeley, 1985; Imdorf, Ruoff & Fluri, 2008). Accordingly, for offspring colonies to survive, swarms need to be large and produced early in the season (Seeley & Visscher, 1985). European honeybees thus invest relatively more resources into their nest and food stores and produce fewer and more costly offspring compared to their tropical relatives (Winston, Taylor & Otis, 1983; Winston, 1991). A major problem of this strategy is that the resources accumulated in the nest, which are highly attractive to all kinds of commensals and depredators (Morse & Flottum, 1997), need to be protected during times of cold temperatures when the bees have limited capacity for active defence. Hence, the ecological prerequisite for honeybees to thrive in temperate climates is the availability of high-quality shelters. Typically, such places are rock crevices or tree cavities. To find the perfect home, scout bees search a vast area and evaluate their findings using a whole catalogue of criteria, the most important ones being size (approximately 10 L as the absolute minimum) and protection (i.e., a small entrance better than a large entrance) (Seeley & Morse, 1978; Seeley, 2019). After hours or days, the result of an elaborate process of collective decision-making is that the scouts reach a consensus over the best cavity and the swarm moves in (Seeley, 2010). The bees clean the interior and apply propolis (a mixture of plant resin and beeswax) to the cavity walls as protection against mould and pathogens, and they successively fill the entire cavity with several sheets of beeswax combs in which to raise brood and store pollen and honey (Seeley & Morse, 1976). Given these investments, there is little reason for colonies to move again; they stay at one nest site for their whole life. Their need for high-quality cavities, their sedentary lifestyle, and their tendency to create large honey stores are all factors that favoured the development of apiculture with European honeybees.

Traditional forms of beekeeping

We can assume that humans have always hunted for the nests of wild honeybees, since honey is among the sweetest and most caloric foods naturally encountered. With the rise of large civilisations in Mesopotamia and Egypt, people also learned to establish bee colonies at their homes, making the harvest of honey and beeswax a less dangerous and more prolific business (Crane, 1999). The simple methodological basis of beekeeping is the provision of artificial cavities – hives – that suit the bees' needs. Traditional beehives are simple vessels in which honeybees directly anchor their natural comb nest ("fixed-comb hives"): pots of clay, cylinders

of mud, skeps of straw, tubes of hollowed tree trunks, or simple boxes of wooden boards. The fundamental beekeeping skills were the crafting of hives, the capturing of swarms, the mastering of smoke (to keep bees from stinging), and the harvesting and processing of hive products. In central Europe, beekeeping with skeps or upright log hives was practised from the beginning of the 1st century AD (Crane, 1999). From an eco-evolutionary perspective, the rise of what we now call "traditional" apiculture might have led to an increase in the size of local honeybee populations (due to an increase in the availability of nest sites) and a more clustered spatial distribution of colonies (due to the crowding of hives in apiaries). However, the biology of the bees remained basically unaltered because there was little room for manipulating honeybee colonies living in fixed-comb hives.

Aside from apiculture with hives, systematic tree beekeeping developed in forested regions as a direct descendant of honey hunting (Crane, 1999). A tree beekeeper creates cavities in large trees that are then spontaneously occupied by swarms and inspects the cavity for non-destructive honey harvest and maintenance (Schirach, 1774). Like beekeeping with hives, which has also been referred to as "house beekeeping" or "garden beekeeping", tree beekeeping, also known as "forest beekeeping", supported honeybees by provisioning them with additional nest sites. However, in the latter type of bee culture, the life of the bees was even closer to nature, because the artificial cavities resembled natural ones in terms of location and distribution. Furthermore, tree beekeeping itself depended on the availability of natural honeybee swarms, so the practice can be regarded as an indicator of the existence of viable wild honeybee populations in forests.

Tree beekeeping was mainly practised in the large forests of eastern and east-central Europe, the Nuremberg State Forest in Germany being the westernmost hotspot (Crane, 1999). From the 12th century on, the harvest of bee colonies in tree cavities was an important source of honey and wax in Central Europe (Ruttner, 1992). However, by the early 18th century, tree beekeepers struggled to find the mature trees needed to create cavities and/or were denied the right to use them (Schirach, 1774). This is because the overexploitation of forests or their complete conversion to agricultural land led to a decline in the density of large trees, and the implementation of regulated forestry banned other forms of woodland use (Küster, 1998). The consequence was an almost complete shift from tree beekeeping in woodlands to hive beekeeping in the agro-urban space (Ruttner, 1992; Banaszak, 2009). The end of historical records on honey and wax production by tree beekeeping marks the end of historical evidence of large wild honeybee populations in European forests.

Modern apiculture with movable-frame hives

The comprehensive shift to beekeeping in hives in central Europe meant an ecological change but not a biological one for the bees. Initially, apiaries were still dominated by traditional fixed-comb

hives in which the bees had control over their reproduction. This started to change only in the 1850s, when a series of inventions led to the worldwide adoption of movable-frame hives (Crane, 1999). Modern beehives share three key features that revolutionised apiculture by enabling beekeepers to manipulate the development of honeybee colonies in favour of colony growth and honey production and against natural reproduction (Seeley, 2019). First, modular hive bodies made it possible to flexibly adjust a colony's nesting space. Second, rectangular wooden frames, inside which the bees neatly built their wax combs when correctly dimensioned and spaced, allowed beekeepers to move individual combs without destroying the nest. And third, wax foundations (thin sheets of beeswax containing a blueprint of hexagonal cell patterns) enabled beekeepers to control basic nest architecture. In cavities of fixed volume, honeybee nests quickly become crowded, which triggers preparation for drone (male bee) production and swarming in spring (Loftus, Smith & Seeley, 2016; Smith, Koenig & Peters, 2017). By stacking additional boxes and honey supers on top of the hive, beekeepers provide additional nest space, which delays swarming and promotes colony growth and the storage of surplus honey(Rinderer & Baxter, 1978; Seeley, 2019). In preparation for fission, honeybee colonies produce several new queens in cone-like queen-rearing cells, since a young queen needs to take over the nest after the old queen and other young queens have left with swarms (Winston, 1991). With combs in movable frames, however, the whole nest can be inspected, and the queen-rearing cells can be removed preventively, inhibiting the swarming process. For drones to be reared, honeybee colonies build special drone comb with cells that are larger than the cells of the worker comb. Natural nests contain around 20% of drone comb and colonies produce thousands of drones in one season but, with the use of worker cell wax foundation in frames, drone production is effectively controlled because there is little room for drone cells (Allen, 1965; Seeley & Morse, 1976; Kohl et al., 2015). Keeping male bees out of the nest also benefits honey production because honeybee drones consume a lot of food but do not forage for themselves (Seeley, 2002). The movable frame hives did not only enable control over the natural swarming behaviour and drone production; they also allowed for the deliberate artificial multiplication of colonies by splitting nests into so-called nucleus colonies. However, due to the peculiarities of the mating behaviour of honeybees, the new opportunities to steer colony development did not include control over the male bees with which queens have offspring. The latter mate in the air with multiple drones (typically 10–20) during one or several nuptial flights within the first few weeks of their lives (Koeniger & Koeniger, 2014). They avoid inbreeding by mating at distances up to 15 km from the hive, beyond the typical dispersal range of their brothers (about 2 km), at so-called drone congregation areas, aerial mating leks, where drones from multiple foreign colonies assemble (Rowell, Taylor & Long-Rowell, 1992; Baudry et al., 1998; Jensen et al., 2005; Koeniger, Koeniger & Pechhacker, 2005; Woodgate et al., 2021). The random mating with multiple unrelated males that are unlikely to stem from the same home site (apiary) maximises the genetic diversity among the queens'

future worker offspring and essentially impedes selective breeding within individual beekeeping operations (Moritz & Crewe, 2018).

Introduction of allochthonous honeybees

The first profound genetic alterations of honeybee populations were not due to artificial selection at apiaries but to the introgression of genes from allochthonous honeybee populations (Rothenbuhler, 1958; De la Rúa et al., 2009). New possibilities of long-distance travel and trade via railway arising in the 19th century made it possible to import non-native honeybee colonies (Ruttner, 1992). Within Europe, the main pattern of honeybee trade was that Italian honeybees (*A. m. ligustica*) and Carnolian honeybees (*A. m. carnica*), both belonging to the C evolutionary lineage, were introduced into the territories of the M-lineage dark European honeybees (*A. m. mellifera*) north and west of the Alps (Crane, 1999). According to Ruttner (1992), the wish of beekeepers to exchange their stock was mainly driven by novel demands created by movable-frame hives and the growing importance of early-flowering crops like oilseed rape as a nectar source in agricultural landscapes. Typical traits of *A. m. mellifera* include nervous behaviour, the ample collection of propolis and a slow increase in brood production in spring, while both *A. m. ligustica* and *A. m. carnica* are naturally characterised by relative calm and gentleness, moderate use of propolis and rapid colony development. Handling combs in frames is more comfortable when they are not firmly attached to the hive walls with propolis, and inspecting colonies is easier with calm workers than with bees that quickly leave the combs and ball at the corners of the frame (as *A. m. mellifera* supposedly does) (Ruttner, Milner & Dews, 1990; Ruttner, 1992). Lastly, the potential of modern hives in creating large colonies for honey production is more conveniently exploited when colonies produce plenty of brood early in the season. Together, these factors mean that C-lineage bees happened to be better adapted to beekeeping with the new types of hives (Ruttner, 1992). Since there is no clear reproductive barrier between subspecies and there was no mating control by beekeepers, hybridisation readily happened and the honeybee populations in Central Europe north of the Alps became heavily introgressed until the middle of the 20th century (Moritz, 1991; Soland-Reckeweg et al., 2009). Retrospectively, the hybridisation of M-lineage and C-lineage honeybees can be regarded as dramatic in evolutionary terms because we now know that the two lineages had been naturally separated for about four to six million years (Dogantzis et al., 2021). From a nature conservation perspective, the practical disappearance of pure-bred *A. m. mellifera* in Central Europe is obviously regrettable, but the widespread hybridisation also had negative consequences for practical beekeeping. Even though first-generation hybrids of subspecies can show above-average productivity through heterosis effects, subsequent generations vary widely and often show undesirable traits like extreme defensiveness (Ruttner, 1992).

The phase of uncontrolled introduction and crossbreeding was superseded by an era of systematic promotion of pure subspecies. Starting in about 1950, beekeeping associations and bee research institutes in Germany initiated the world's largest honeybee breeding programme aiming to replace the native dark bees and their hybrids with unhybridised *A. m. carnica* with stable or improved characteristics (Moritz, Härtel & Neumann, 2005; Mittl, 2019). Through advances in the artificial rearing of honeybee queens and the understanding of their mating biology (Ruttner, 1988b; Büchler et al., 2013; Koeniger & Koeniger, 2014), the breeding efforts succeeded in that, today, Carnolian-derived honeybee queens dominate apiaries in Central Europe (Reinsch et al., 1991; Kauhausen-Keller & Keller, 1994; Francis et al., 2014). Through the mass-rearing and distribution of queen offspring from best-performing colonies and the usage of isolated mating apiaries, traits important to beekeeping like honey yield, gentleness, low propensity to swarm and "comb-steadiness" gradually improved in several breeding lines (Hoppe et al., 2020).

Obstacles to the domestication of honeybees

The varied apicultural inventions and breeding efforts of the last 170 years have not only changed how honeybees are kept by beekeepers, but they have also contributed to the adoption of the western honeybee as a biological model organism for animal behaviour, physiology, genetics, and ecotoxicology (von Frisch, 1967; Weinstock, 2006; Galizia, Eisenhardt & Giurfa, 2012). For example, combs in movable frames have made it easy to install colonies in small glass-windowed hives for behavioural observations, and the availability of gentle Carnolian queens has facilitated the handling of bees for biologists (von Frisch, 1967; Crane, 1999). Given the special attention that bee associations devote to selective breeding and the boundless possibilities of manipulating the bees with movable combs, there seems not to be much "wildness" left within the honeybee. However, our day-to-day experiences heavily overestimate the degree to which *A. mellifera* is biologically altered by, and dependent upon, humans. Unless queens are artificially inseminated, which is rarely done as it is a laborious task requiring special skills, there is no complete mating control (Moritz & Crewe, 2018). Due to their vast mating areas, even at seemingly isolated mating apiaries on islands, queens also mate with drones other than those of the selected colonies (Neumannu et al., 1999). Therefore, artificial selection with honeybees mainly affects the female side of reproduction, explaining why the "improvement" of traits is a such tedious process (Moritz & Crewe, 2018). While the matriline is easy to control in breeding programmes, the fact that honeybees from Germany still exhibit a strong influence from the native dark bee shows the considerable impact of unselected drones on the nuclear genome (Moritz, 1991). As a matter of fact, these obstacles to breeding honeybees are advantageous for beekeeping. The main task of the bees living in apiaries is still the same as it was millions of years ago. While we closely control the food that other livestock species feed on and where they do it, honeybees are exploited for their natural behaviour of collecting nectar and pollen from flowers, a substantial portion of which

stems from wild plants. Since they forage freely within a large area of up to 5–10 km around their nests (Visscher & Seeley, 1982; Steffan-Dewenter & Kuhn, 2003; Rutschmann, Kohl & Steffan-Dewenter, 2023), they are heavily influenced by local environmental conditions. Therefore, the performance and survival of colonies still depend to a substantial degree on their adaptation to the local environment (Louveaux et al., 1966); the uncontrolled mating of their queens with local drones increases the chances of being well-adapted. The importance of local adaptations was famously illustrated by a Europe-wide collaborative experiment in which bee research institutes exchanged colonies to see whether any subspecies or breeding lines are superior (Büchler et al., 2014). Strikingly, regardless of evolutionary history, colonies placed at their apiary of origin significantly outperformed non-native colonies in survival rate (Büchler et al., 2014). Consequently, selected colonies that perform well at one location are not necessarily superior at a different one, and the wide dissemination of queens produced in breeding programmes is unlikely to be sustainable.

Another factor that is typically neglected in bee breeding is that there have always been honeybees living in wild nests beyond apiculture. For example, in an amendment to a monograph on the bee fauna of Franconia, Stoeckhert reports on observations of wild-living honeybee colonies in different forests in southern Germany, which led him to the hypothesis that they might be more common than he had previously assumed (Stoeckhert, 1933, 1954). Since naturally-nesting colonies that have survived a winter in a tree cavity will typically produce many drones in the following spring, such colonies probably have some influence on the gene pool of regional honeybee populations, passing on genes that help colonies survive under wild conditions. Ultimately, an important argument for honeybees not being properly domesticated is that colonies can quickly revert to a natural state. When left unsupervised, honeybee colonies will still swarm in spring, skilfully select a nesting cavity, and build a natural comb nest without any support from a beekeeper (Seeley, 2010). Instead of being genetically domesticated on the population level, every individual honeybee colony and its extended phenotype, the nest of beeswax comb, needs to be *tamed* by beekeepers using various apicultural tools and techniques (Seeley, 2019).

A novel parasite as a game changer for wild-living honeybees?

The hybridisation of different subspecies and artificial selection certainly affected honeybee populations to some degree (Lecocq, 2018; Hoppe et al., 2020; Themudo et al., 2020), but these factors did not lead to a critical dependency of the bees on humans – they essentially remained wild animals (Moritz & Crewe, 2018; Seeley, 2019). The latest human-mediated impact, however, seems to have made the species vulnerable. When western honeybees were imported to Asia for honey production and pollination services, they started to share their habitat with the closely related Eastern honeybee, *Apis cerana*. The latter lives in a stable host-parasite

relationship with ectoparasitic mites of the genus *Varroa,* which reproduce in brood cells and feed on the haemolymph and fat body of larvae and adult bees. At some point in the 1950s, western honeybee colonies also became infested with mites of the species *V. destructor* (reviewed by Traynor et al., 2020). Unfortunately, *A. mellifera* had no evolved defence mechanism against them, so the mites' reproduction was unhindered. Aided by the worldwide trade in bees, migratory beekeeping, and the crowding of hives in apiaries, the new parasite spread quickly and had invaded most continents by the end of the century, with central Europe being colonised in the 1970s and 80s (Traynor et al., 2020). Apart from directly damaging the bees by feeding on them, the mites acted as a novel vector for pathogens like deformed wing viruses and caused a worldwide health crisis of managed honeybee colonies (Di et al., 2016; Wilfert et al., 2016). Until today, beekeepers are urged to control mite populations by regularly treating their hives with acaricides (Bartlett, 2022).

But what was the effect of *V. destructor* on honeybees living in wild nests? Since managed colonies usually die within a couple of years when left untreated (Rosenkranz, Aumeier & Ziegelmann, 2010), the belief spread among beekeepers and researchers that wild-living honeybees were wiped out by the mite (Thompson et al., 2014; Meixner, Kryger & Costa, 2015). While it is reasonable to assume a negative effect on wild-living colonies (Kraus & Page, 1995), a literature search for direct evidence of a wild population decline following the *V. destructor* invasion in Europe revealed that there is no such data. Interestingly, statements by bee scientists made decades before the arrival of the mite indicate that wild-living honeybees were already considered extinct in the middle of the 20th century (Stoeckhert, 1933; Zander, 1944 cited by Mittl, 2019). Back then, the decisive factor for their disappearance was thought to be the lack of nesting cavities in managed forests. However, such judgements seemed to have not been based on any data either. In Europe, wild-living honeybees had simply never been specifically investigated (Kohl & Rutschmann, 2018).

Wild-living honeybees have probably been underrepresented in bee research because topical questions regarding fundamental bee biology were readily answered using colonies in hives, and only managed honeybees seemed economically and ecologically important. The situation is different outside of Europe. Especially in the introduced range of the species in the Americas and Australia, scientific studies on wild-living honeybees date back at least to the 1970s. European honeybees were brought to North America in the early 17th century and to Australia about 200 years later and, on both continents, their swarms quickly established large wild populations (Ruttner, 1992; Crane, 1999). In Australia, much of the research interest in wild-living honeybees is explained by their role as competitors of the native fauna for nest sites and floral resources (Paton, 1996; Cunningham et al., 2022). However, honeybees living in temperate deciduous forests in the northeastern USA – in a habitat that resembled their original one – served as subjects

to study the basic ecology of European honeybees in the wild (Seeley, 2019). Our current scientific knowledge about natural honeybee nests in tree cavities, their nest-site preferences and their life-history strategy is mainly based on the pioneering studies of T. D. Seeley and his colleagues conducted in the Arnot forest in New York State (reviewed by Seeley, 2019). Data on colony densities and colony survival rates were already recorded in the 1970s and 1980s, before *V. destructor* invaded North America, serving as a baseline reference for quasi-natural conditions (Seeley, 1978; Visscher & Seeley, 1989). Strikingly, reanalyses of population demography after the arrival of the mite showed that the wild-living honeybees still occurred at densities of about one colony per km^2 and that they still formed a stable population with virtually the same life-history characteristics (i.e., colony longevity, reproductive rate) as in the 1970s (Seeley, 2007, 2017). These results raised the question of which environmental and/or genetic factors allow these honeybees to persist with *V. destructor,* while managed colonies perish without medical treatment. Genetic comparisons of contemporary wild-living colonies and historical samples showed signatures of rapid evolution upon the arrival of the mite, suggesting that naturally selected defence mechanisms are involved in explaining population stability (Mikheyev et al., 2016). However, observations of the mite population growth in wild colonies also showed that the latter clearly remained vulnerable to the parasite (Seeley, 2007). A key insight obtained from the study of the Arnot forest bees is that environmental differences between wild nests and managed hives at apiaries play a critical role in wild population persistence (Seeley, 2019). It is the peculiarities of their living conditions, including small and widely spaced nests, and their behavioural consequences, like frequent swarming, that allow wild-living colonies to survive on the population level without the need for costly behavioural defences (Seeley & Smith, 2015; Loftus, Smith & Seeley, 2016; Seeley, 2017).

First systematic censuses of wild-living honeybee colonies in Europe

The findings of the Arnot forest bees raised the question of whether wild-living colonies might also be found at other places, and if so, whether they also form stable populations despite infestation by *V. destructor*. Wild honeybee nests are often well hidden high above the ground, and nobody had ever made a systematic search in Europe. A study by Thompson (2012), who investigated wild-living honeybee colonies located through citizen reports in the UK, and the report of Oleksa, Gawroński and Tofilski (2013) on wild-living colonies occupying tree cavities along rural avenues in northern Poland suggested that they are indeed much more abundant than commonly assumed. Based on an article by Visscher and Seeley describing the technique of locating wild nests by means of beelining (Visscher & Seeley, 1989) my colleague Benjamin Rutschmann and I set out to make a first systematic census in Germany (Kohl & Rutschmann, 2018). We found that wild-living colonies can still be found in old-growth beech forests (Figure 1.1). Although the estimated minimum colony density was relatively low compared to the

density of managed hives, wild-living colonies also occurred deep inside the forests, several km away from villages and apiaries. That study marked the starting point for our investigations of wild-living honeybees in Germany and laid the groundwork for this thesis.

Figure 1.1: Wild-living honeybee colony nesting in a black woodpecker (*Dryocopus martius*) cavity in a beech tree on the Swabian Alb (Photo credit: Ingo Arndt).

Why wild-living honeybees deserve our attention

The western honeybee can be readily maintained in hives for honey production and pollination service delivery and the number of managed colonies is rising (Phiri, Fèvre & Hidano, 2022). Furthermore, most of the biological discoveries made with honeybees have been based on colonies nesting in beekeeping or observation hives (e.g., von Frisch, 1967; Seeley, 1995; Galizia, Eisenhardt & Giurfa, 2012). One might wonder, then: what are the scientific arguments for the study and conservation of the honeybee in the wild? In the following, I present two major lines of reasoning. The first focuses on the ecological interactions of wild-living honeybees. The second emphasises the potential value of wild honeybee populations as genetic reservoirs.

Ecologically, wild-living honeybees complement managed honeybees both spatially and functionally. Beekeepers predominantly keep their colonies in urban and agricultural areas; at best, hives are moved into the forest during short periods of the year to exploit the flows of honeydew produced by plant-sucking insects. Hence, if wild-living honeybee colonies did not exist, the pollination services of honeybees to wild plants in their natural woodland habitat would probably be reduced. Furthermore, since bees are usually kept near the homes of beekeepers, the

density of managed colonies might not always meet the need for pollination in sparsely populated rural areas (Chang & Hoopingarner, 1991). The existence of wild-living honeybees nesting in rural avenues and forest fragments could lessen this problem. Functionally, the use of tree cavities as nesting sites adds a whole new dimension of interactions to the ecology of wild-living honeybees compared to managed ones: swarms compete with other cavity nesters for tree holes (Reinsch, 1979; Oldroyd, Lawler & Crozier, 1994; Paton, 1996; Cunningham et al., 2022), the bees and their nests are important resources for other insects, birds, and mammals (Morse & Flottum, 1997), and, by lining cavity walls with antimicrobial plant resins and accumulating biomass (Seeley & Morse, 1976; Simone-Finstrom & Spivak, 2010), wild-living colonies probably influence the community composition of organisms that depend on the decaying matter in tree cavities. These interactions are almost completely excluded from managed hives and have been poorly explored.

Regarding the conservation of the honeybee, apiculture guarantees the maintenance of colony numbers, but beekeeping alone is unlikely to preserve the genetic diversity within the species (Requier et al., 2019a; Panziera et al., 2022). Due to international trade in bees and the preference of beekeepers for a subset of lineages, native honeybee populations and their unique genetic combinations are endangered through direct displacement and introgressive hybridisation (De la Rúa et al., 2009; Themudo et al., 2020). Furthermore, regardless of the geographic origin of the bees, traits relevant to beekeeping that are artificially selected in breeding programmes, e.g., increased gentleness and decreased propensity to swarm, are most likely maladaptive under natural conditions. In fact, the laboriously selected breeding lines are often maintained under high rates of management input, raising questions about sustainability and the long-term conservation of the honeybee. Populations of wild-living honeybees, in turn, can function as genetic reservoirs of native subspecies or new locally adapted populations (Mikheyev et al., 2016; Pirk, Crewe & Moritz, 2017).

Importantly, fostering wild-living honeybees would not only benefit biodiversity. The scientific insights obtained by studying their ecology, and the genetic resources they harbour (or which are allowed to evolve within wild populations), can be sources of both knowledge and genetic material for a future sustainable apiculture that works with local bees and without medical treatments (Neumann & Blacquière, 2017; Seeley, 2019).

A framework for the investigation of the honeybee in the wild

In Europe, the concept of the honeybee as a wild animal often creates confusion because a prevailing view among beekeepers, researchers, and conservationists is that the species entirely

depends on maintenance by humans. Before diving into the case studies, it is therefore crucial to reflect on some premises, define ambiguous terms, and highlight the outstanding questions.

Historically, honeybees were referred to as either "house bees" or "forest bees" depending on whether they lived in apiaries near settlements or in tree cavities in the forest (Schirach, 1774). No difference was made between "domesticated" and "wild" honeybees, as these categories do not suit the biology of the species (Ruttner, 1992). Honeybee queens and drones naturally mate in a vast area around their nests, meaning that all colonies in a region – regardless of whether they live in beekeeping hives – generally belong to one biological population (Rowell, Taylor & Long-Rowell, 1992; Jensen et al., 2005). Consequently, the reproduction of honeybees kept in apiaries has never been completely controlled and the species is not domesticated in the sense that applies to many livestock or companion animals (Moritz & Crewe, 2018). Since the attributes "domesticated" and "wild" suggest profound differences in evolutionary history, they are misleading when used to describe honeybee colonies. Instead, it is more appropriate and practical to refer to the bees' current management status and to distinguish between *managed* and *wild-living* (or *free-living*) colonies. The latter are defined as colonies that are ownerless and live in cavities they have chosen and occupied themselves.

A key step for the investigation of wild-living honeybees is to take on a population perspective (Oldroyd et al., 1997; Seeley, 2017). This is a fundamental difference from the focus of apiculture, where the typical unit of interest is the individual colony. Here, a basic (often unconscious) premise is that each colony is potentially immortal: the bees naturally replace old queens with daughter queens (or obtain a new artificially reared queen by the beekeeper), and the nest is prevented from ageing by the successive replacement of old comb frames with empty frames containing wax foundation. Due to this perspective, beekeepers devote substantial resources to supporting the growth and survival of individual colonies with the aim to achieve 100% colony survival each year. At the same time, colony reproduction is heavily reduced by preventing swarming. As a logical consequence, any stressor that leads to a reduction in colony survival rate is a potential threat that needs to be mitigated by new management strategies. The most famous example is the invasive parasite *V. destructor,* which causes a clear reduction of a managed colony's lifespan when not treated with acaricides (Rosenkranz, Aumeier & Ziegelmann, 2010; Traynor et al., 2020). As a logical extrapolation of the need for treatment in apiculture, wild-living honeybee colonies are considered not to be able to survive on their own (Rosenkranz, Aumeier & Ziegelmann, 2010; Thompson et al., 2014; Meixner, Kryger & Costa, 2015). However, in natural populations, it is normal that organisms die, and that reproduction compensates for mortality (Loper et al., 2006; Villa et al., 2008; Seeley, 2017). Therefore, it needs to be investigated whether wild-living honeybees are persisting at the population level despite individual colonies being vulnerable to parasites.

To study honeybees' population demography, it is practical to consider the cohorts of wild-living colonies and managed colonies as two subpopulations that form a regional metapopulation (the terms *cohort* and *population* are used interchangeably in this context) (Figure 1.2). The size of the wild-living population can change due to migration, and due to the mortality and reproduction of the existing wild-living colonies. "Immigration" occurs when managed colonies swarm and escape from apiaries. "Emigration" theoretically occurs when wild-living colonies are artificially removed from natural nest sites or when their swarms are caught by beekeepers. In Germany, especially in forest areas, the frequency of "emigration" is neglectable because beekeepers usually obtain colonies from other beekeepers and generally do not install bait hives to attract honeybee swarms. Therefore, what remains to be investigated is whether the annual mortality rate of the wild-living colonies can be compensated for by the reproductive swarming rate of the surviving wild-living colonies. If that is the case, the wild-living colonies potentially form a self-sustaining population. If not, their existence is explained by the recurrent immigration of swarms escaped from apiaries.

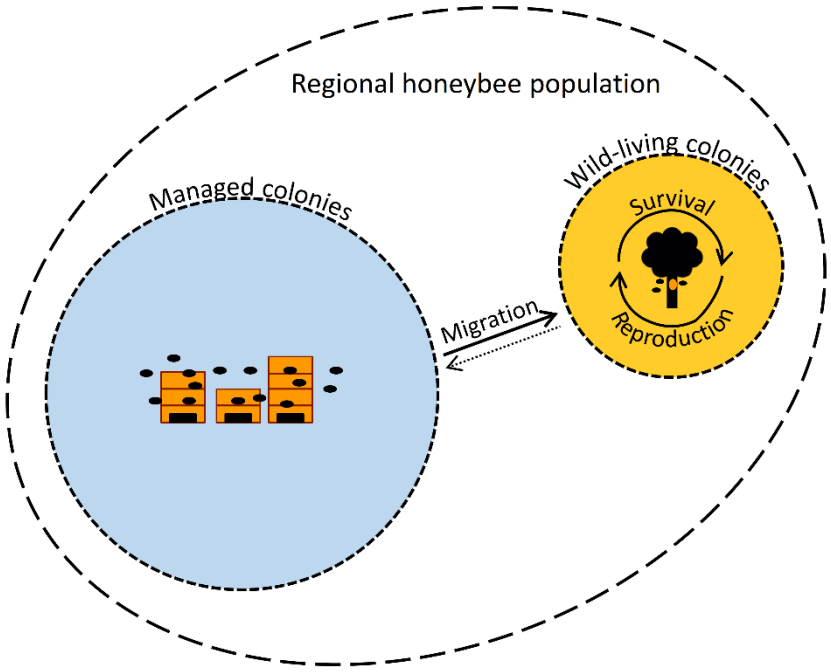

Figure 1.2: Metapopulation model for the study of wild-living honeybee populations. All colonies in a region belong to one biological population due to genetic exchange through the random mating of queens and drones. Colonies can migrate between the cohorts of managed colonies and wild-living colonies.

Given that the average reproductive rate of temperate-adapted honeybee colonies equals about two swarms per colony per year (Seeley, 2019; see supplementary information in chapter one), autonomous population replacement is readily achieved at an average colony survival rate of 33.33%. This number is much lower than the survival rates typically observed in apiculture (70–

100%, Genersch et al., 2010; Chauzat et al., 2013; Johannesen et al., 2022), illustrating that wild-living colonies might not require special adaptations, e.g., parasite resistance, and do not necessarily need to differ genetically from managed colonies to form self-replacing populations. Conversely, the population-level stability of the wild-living colonies is the prerequisite for genetic differences between wild-living and managed populations to be conserved or to evolve. This means that, where managed honeybee populations are heavily influenced by selective breeding, the potential value of wild-living honeybee populations as a genetic reservoir only applies in case they form self-sustaining populations. These considerations highlight the importance of collecting demographic data as the first step in any investigation.

Wild-living honeybees of European origin living in the Arnot Forest in the northeastern USA (Seeley, 1978, 2017) and Wyperfield National Park in Australia (Oldroyd et al., 1997) have been investigated in detail with respect to colony survival rates, and it has been found that their populations are self-sustaining. Interestingly, these non-native wild-living honeybees have been, by convention, referred to as "feral bees" (e.g., Paton, 1996), but this is misleading. "Feral" is derived from the Latin word *ferus,* which simply means being wild or untamed, but biologists more specifically use the word to describe animals that have reverted to life in the wild after living in captivity. The process of feralisation can either refer to the phylogeny of whole populations that undergo the evolutionary process of de-domestication (Gering et al., 2019), or to the ontogeny of individual organisms that undergo the behavioural process of escaping from captivity (Daniels & Bekoff, 1989). In the case of honeybees, only the second meaning may apply since a species that is not domesticated cannot be de-domesticated. Given that the populations of wild-living colonies in the Americas and Australia have their roots in the first introductions of honeybees by European settlers centuries ago (Ruttner, 1992; Crane, 1999; Carpenter & Harpur, 2021), calling them "feral" in the behavioural sense does not make sense either. I therefore suggest that any self-sustaining population of wild-living honeybee colonies, regardless of whether the native range of the (sub)species is concerned, qualifies to be referred to as a wild honeybee population (also note the adoption of the term "wild" for wild-living colonies in North America by Seeley, 2019). In turn, I will only use the term "feral" in the behavioural sense (Daniels & Bekoff, 1989) to refer to wild-living colonies that have recently escaped management or, analogously, to populations of wild-living colonies that are not self-sustaining.

Besides the realisation that relatively low colony survival rates can be sufficient for honeybee population stability, an important premise of this work is that the cohorts of wild-living and managed colonies differ in several functionally relevant aspects of their environments (Seeley, 2019). These environmental differences, in turn, determine which factors limit colony survival. For example, a major driver of colony mortality in managed honeybees is parasite pressure (Brosi et al., 2017; Bartlett, 2022), but there is evidence that wild-living colonies are less likely to

develop high parasite loads than managed colonies (Bailey, 1958; Ratnieks & Nowakowski, 1989; Fries & Camazine, 2001). Due to restricted cavity volumes and a lack of young queen removal by beekeepers, wild-living colonies swarm more frequently. Swarming, in turn, leads to temporal brood pauses, which cuts off the reproduction of parasites that depend on the brood (Loftus, Smith & Seeley, 2016; Gabel, Scheiner & Büchler, 2023). Furthermore, the nests of wild-living colonies are more dispersed in the landscape than managed colonies, so the likelihood of inter-colony parasite transmission is reduced (Seeley & Smith, 2015; Nolan & Delaplane, 2017). The possibility of individual honeybee colonies being less prone to developing high parasite loads when living wild than when managed in apiaries is another argument for why wild-living populations might persist despite infestation by *V. destructor* (Seeley, 2007; DeGrandi-Hoffman, Ahumada & Graham, 2017). Other environmental differences can complicate the lives of wild-living colonies. For example, without supplemental sugar feed by beekeepers, wild-living colonies entirely depend on the availability of natural food sources and are thus more likely to die from hunger than managed colonies.

Besides the investigation of the population status of wild-living honeybee colonies in Germany, this thesis investigates their parasite burden. It also offers a first exploration of how much parasite pressure contributes to colony mortality in relation to other environmental factors that rarely affect colonies managed in apiaries.

The study system

Studying wild-living honeybees typically requires information on the dwelling places of colonies and, to obtain quantitatively meaningful data, many nest sites need to be known. This represents a major challenge since wild-living honeybee colonies are difficult to find. Especially in forests, the abundance of potential nesting trees and the jumble of leaves, light and shadow make it improbable to spot the bees' nesting cavities based on random search alone. A way of informed search is "beelining", whereby the homing flights of bees foraging at a sugar bait are followed to their nests (Visscher & Seeley, 1989; Seeley, 2016). However, beelining is time-consuming, and even if a narrow area containing the nest site has been determined, it can still be hard to determine where exactly the cavity entrance is. I learned this during a pilot bee hunt in the forest of Gramschatz in August 2016. In the afternoon of the second beelining day, I had narrowed down the nest site to a quarter of a hectare of forest. But it was only after several hours of scanning tree trunks with binoculars, just before giving up, that I got a glimpse of bee traffic glittering in the light of the evening sun at a mature beech tree at about 15 m height. The cavity entrance was not directly visible, however, since the view was blocked by an umbrella of leaves. Only upon returning to the place in autumn, when the leaves had fallen, could I verify its existence. B. Rutschmann and I had a similar experience during a systematic beelining census in the beech

forest of the Hainich National Park. Once we had followed the bees close to their nests into the deep forest, it became difficult to obtain readings of their home flight directions because they simply vanished in the canopy. We could therefore only map bee trees with an uncertainty of about 100–200 m (Kohl & Rutschmann, 2018).

Our solution to the problem of finding wild honeybee nests at an acceptable rate is based on a report on the communities of animals using tree cavities excavated by the black woodpecker (*Dryocopus martius*) (Sikora, Schnitt & Kinser, 2016). We learned that black woodpeckers function as key ecosystem engineers because they create cavities with relatively large volumes (typically around 10 L, Kosiński & Walczak, 2019) without depending on rotten trees (Zahner, Sikora & Pasinelli, 2012). In managed forests, where trees rarely develop holes by damage and decay (Remm & Lõhmus, 2011), their cavities are the primary nesting sites for a range of secondary cavity users, including honeybees (Johnson, Nilsson & Tjernberg, 1993; Kosiński et al., 2010; Sikora, Schnitt & Kinser, 2016). Luckily, the systematic mapping of black woodpecker cavities is increasingly used as a tool for nature conservation in managed forests, so there are districts with near-exhaustive lists of cavity trees (Sikora, 2009; Bütler et al., 2020). During our first inspection of about one hundred known cavity trees in beech forests of the Swabian Alb in September 2017, we found seven wild-living colonies within less than one week of fieldwork – a discovery rate far higher than that which can be achieved with beelining. The idea that black woodpecker cavities are the primary nesting sites for honeybee colonies in German forests was supported by the notion that the unbiased sampling method of beelining yields similar colony density estimates as the direct inspection of woodpecker cavities (Kohl & Rutschmann, 2018). Therefore, we assumed that focusing solely on woodpecker cavities would produce a representative sample of forest-dwelling honeybee colonies. The work presented in this thesis is primarily based on wild-living honeybee colonies nesting in black woodpecker cavities in managed forests in three rural study regions in southern Germany: the Swabian Alb in the state of Baden-Württemberg, the counties Coburg and Lichtenfels in the north of the state of Bavaria, and the county Weilheim-Schongau in the south of Bavaria.

When wild-living honeybee colonies are mapped in non-forest areas, other strategies of nest site search can be used. In cities, green belts and parks often contain mature trees with cavities, and beelining and inspecting trees is easier there than in forests because trees are more widely spaced. Furthermore, engaging the public readily yields colony sightings in urban areas (Thompson et al., 2014; Browne et al., 2021; Dubaić et al., 2021). For example, in the city of Munich, the combination of citizen reports and private searches by S. Roth and F. Remter led to the registration of more than 80 nest sites over the past few years. For one of our studies (chapter three), we additionally considered wild-living honeybee colonies nesting in these listed cavities in trees or building walls in the city of Munich.

Outline of the thesis

In the following three chapters (chapters two to four) I present three original studies in the form of standard articles, which I have conducted with different co-authors, and which have been, or will be, published alongside this thesis in scientific journals (Kohl et al., 2022; Kohl, Rutschmann & Steffan-Dewenter, 2022). Chapter two reports on a monitoring study that answers the question of whether the wild-living honeybee colonies occupying woodpecker cavities in German forests form a self-sustaining cohort, or whether their existence is explained by the recurrent emigration of honeybee swarms from managed apiaries. Chapter three deals with disease ecology. It compares the parasite burden of wild-living colonies with that of managed colonies to answer the applied question of whether the former could play a role as reservoirs or vectors of bee parasites. Chapter four combines data on colony survival with data on parasite burden, as well as with data on nest depredation and landscape context. It asks whether any one of the three factors parasite pressure, nest depredation or landscape-level food availability is a likely driver of winter mortality of the wild-living honeybee colonies living in German forests. I conclude with a general discussion (chapter five), which expands on the discussions presented within each of the data chapters.

Chapter two

Population demography of feral honeybee colonies in central European forests

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Abstract

European honeybee populations are considered to consist only of managed colonies, but recent censuses have revealed that wild/feral colonies still occur in various countries. To gauge the ecological and evolutionary relevance of wild-living honeybees, information is needed on their population demography. We monitored feral honeybee colonies in German forests for up to four years through regular inspections of woodpecker cavity trees and microsatellite genotyping. Each summer, about 10% of the trees were occupied, corresponding to average densities of 0.23 feral colonies per km^2 (an estimated 5% of the regional honeybee populations). Populations decreased moderately until autumn but dropped massively during winter, so that their densities were only about 0.02 colonies per km^2 in early spring. During the reproductive (swarming) season, in May and June, populations recovered, with new swarms preferring nest sites that had been occupied in the previous year. The annual survival rate and the estimated lifespan of feral colonies (N = 112) were 10.6% and 0.6 years respectively. We conclude that managed forests in Germany do not harbour self-sustaining wild-living honeybee populations, but they are recolonized every year by swarms escaping from apiaries.

Introduction

Honeybees are among the most widely known insects due to their timeless cultural and economic value as a source of honey and wax (Crane, 1999). As generalist flower visitors, they are pollinators of many wild and cultivated plants and managing their colonies is crucial for industrial crop production (Leonhardt et al., 2013; Hung et al., 2018; Rollin & Garibaldi, 2019). Today, *Apis mellifera* L. is usually seen as a domesticated species which needs to be maintained by humans to provide these services (Lecocq, 2018). What is largely neglected in both science and practice, however, is that an unknown fraction of its global population is still made up of wild or feral colonies (Pirk, Crewe & Moritz, 2017; Kohl & Rutschmann, 2018). Wild-living honeybee colonies can complement managed ones both in providing ecosystem services (e.g., pollination; Chang & Hoopingarner, 1991; Stanley, Msweli & Johnson, 2020) and disservices (e.g., competition with other species for food and nest sites; Paton, 1996; Herrera, 2020) so they should be considered in population censuses (Jaffé et al., 2010). Furthermore, wild-living honeybee populations can be a reservoir of native and/or locally-adapted genes and therefore deserve conservation (Moritz, Härtel & Neumann, 2005; Oleksa, Gawroński & Tofilski, 2013; Alaux, Le Conte & Decourtye, 2019; Requier et al., 2019a; Browne et al., 2021; Panziera et al., 2022). Finally, studying the life of honeybees in the wild can help understand basic aspects of the species' ecology, which in turn can be relevant for apiculture (reviewed by Seeley, 2019).

The Western honeybee is native to Africa, Western Asia, and Europe, and has been introduced to most other parts of the world (Moritz, Härtel & Neumann, 2005; Dogantzis et al., 2021). In Africa, wild colonies are known to outnumber managed ones (Dietemann, Pirk & Crewe, 2009), and after their introduction in 1956, wild-living African honeybees also rapidly spread throughout (sub)tropical America (Winston, 1992; Calfee et al., 2020). In Europe and Western Asia, in contrast, wild honeybee populations are considered extinct due to the invasion of the ectoparasitic mite *Varroa destructor* and its associated viruses (Thompson et al., 2014; Meixner, Kryger & Costa, 2015). Since no longitudinal population studies have been conducted in Europe itself, this assumption is based on studies of wild European honeybees within the species' introduced range in North America which demonstrated initial drops in population sizes following the introduction of *Varroa destructor* (Kraus & Page, 1995; Loper et al., 2006; Villa et al., 2008). However, it is not clear whether the parasite inevitably causes naïve wild-living honeybees to go entirely extinct because, on the population level, frequent reproduction by established colonies might level out colony losses (Loper et al., 2006; Villa et al., 2008; Seeley, 2017). There is a growing number of reports from Europe documenting the occurrence of honeybee colonies nesting wild in various types of cavities and habitats (Oleksa, Gawroński & Tofilski, 2013; Fontana et al., 2018; Kohl & Rutschmann, 2018; Requier et al., 2020; Browne et al., 2021; Dubaić et al., 2021; Moro et al.,

2021; Oberreiter et al., 2021; Rutschmann et al., 2022), but we currently lack detailed studies of their population dynamics.

To gauge the relevance of these wild-living honeybees, two basic questions need to be answered. On the one hand, we need robust information on their colony densities, how they vary between seasons and years, and how they relate to the densities of managed colonies. This is necessary to estimate how frequently they interact with other organisms and thus how much they matter for ecosystems (Saunders et al., 2021). On the other hand, we need to know whether wild-living honeybee colonies form self-sustaining populations or whether they are instead regularly founded by swarms that emigrate from apiaries (Requier et al., 2019a). Self-sustaining wild populations would be interesting subjects for the study of how honeybees manage to persist despite pressure by parasites (Conte et al., 2020; Grindrod & Martin, 2021). Furthermore, knowing their population demography is necessary for rating the relative importance of apiculture versus near-natural habitat (e.g. woodland) in maintaining honeybee populations and their pollination services (Requier et al., 2020; Rutschmann et al., 2022).

Answering the question of whether a given population is self-sustaining requires information on the annual survival and natality rates of its members (Krebs, 2014; Millon et al., 2019). Temperate-adapted honeybee colonies are sedentary and perennial cavity-nesters. They reproduce via colony fission in spring when the old queen and a number of young queens leave with swarms (daughter colonies) and the old nest is taken over by another young queen. Most wild-living colonies will reproduce annually starting after their first successful hibernation, with the average natality rate being around two swarms per colony per year (Seeley, 2019). Bees from managed colonies can also swarm and enter the wild-living population. Hence, a wild-living population can only be considered self-sustaining if the annual colony survival rate is high enough that losses can be compensated for by new swarms produced by the surviving wild-living colonies.

To determine the annual survival rate, one needs to make repeated surveys of their nest sites (Seeley, 1978, 2017; Oldroyd et al., 1997). Unfortunately, finding an adequate number of colonies is often a cumbersome task. While “bee-lining”, the tracing of honeybees from artificial feeding sites to their homes, can be used as an unbiased search method (Seeley, 2016), finding actual nests (and not only their approximate locations) is very time-consuming (Seeley, 2016; Kohl & Rutschmann, 2018; Radcliffe & Seeley, 2018; Oberreiter et al., 2021). Hence, the most used method is asking the public for help (Seeley & Morse, 1976; Thompson et al., 2014; Youngsteadt et al., 2015; Browne et al., 2021; Dubaić et al., 2021; Moro et al., 2021). The downside of citizen science is that the reported colonies are typically scattered over a large area, so that researchers further rely on many volunteers to collect data on survival rates, potentially compromising data quality. Moreover, honeybee nest sites detected via human search will naturally lead to a bias

towards urban areas, where the densities of beekeeper-managed colonies are usually high (Dubaić et al., 2021). However, wild-living honeybee colonies are ecologically more interesting in natural areas remote from human settlements. Often, these involve forests. Here we present a demographic study of wild-living honeybee colonies in German managed forests based on repeated surveys of cavity trees over a period of up to four years. We capitalized upon current maps of woodpecker cavity trees which are under protection from logging. Monitoring these trees allowed us to infer the regional densities and the temporal population dynamics of wild-living colonies and to determine their annual survival rates. These data, in turn, revealed whether the population was either self-sustaining or immigration-dependent.

Material and methods

Study areas and cavity trees

In forests managed for timber production, trees are rarely allowed to grow old enough to develop large holes through damage or decay (Remm & Lõhmus, 2011; Courbaud et al., 2022). Therefore, species that require large tree-holes for nesting usually rely on cavities excavated by woodpeckers (Johnson, Nilsson & Tjernberg, 1993; Kosiński et al., 2010; Remm & Lõhmus, 2011; Sikora, Schnitt & Kinser, 2016). In German forests, black woodpecker (*Dryocopus martius*) cavities probably represent the main nesting opportunity for wild-living honeybees given that our inspection of woodpecker trees and a different, unbiased search method (bee-lining technique) yielded similar estimates of wild-living colony densities for (albeit different) forests (Kohl & Rutschmann, 2018). We therefore assumed that surveying black woodpecker cavities would yield representative samples of the wild-living honeybee populations in managed forests.

We conducted our wild-living honeybee censuses in forests of three regions in southern Germany for which detailed maps of cavity trees (and the corresponding unique geographic coordinates) were available: in the Swabian Alb (Baden-Württemberg; Sikora, Schnitt & Kinser, 2016), in the counties of Coburg and Lichtenfels (north-Bavaria; N. Wimmer, personal communication), and in and around the county of Weilheim-Schongau (Bavarian Alpine Foreland; K. Zeimentz, personal communication) (Figure 2.1a). The forests were either dominated by beech (*Fagus sylvatica*, the species that would naturally dominate most forests in Germany) or by Norway spruce (*Picea abies,* which is planted for timber production). The typical black woodpecker cavity tree was a large beech tree (>98% of the considered cavity trees were beeches) with a diameter at breast height of 55 cm or more (Figure 2.1b). The cavities usually laid 10–12 m (range: 5–18 m) above ground level and had an entrance with a diameter of around 10 cm (range 5–15 cm) (Sikora, 2009, personal observations). We had no information on the specific volumes of the cavities used

in our survey but they probably held at least around 10 L, which is the approximate volume of freshly excavated black woodpecker cavities in beech trees (Kosiński & Walczak, 2019).

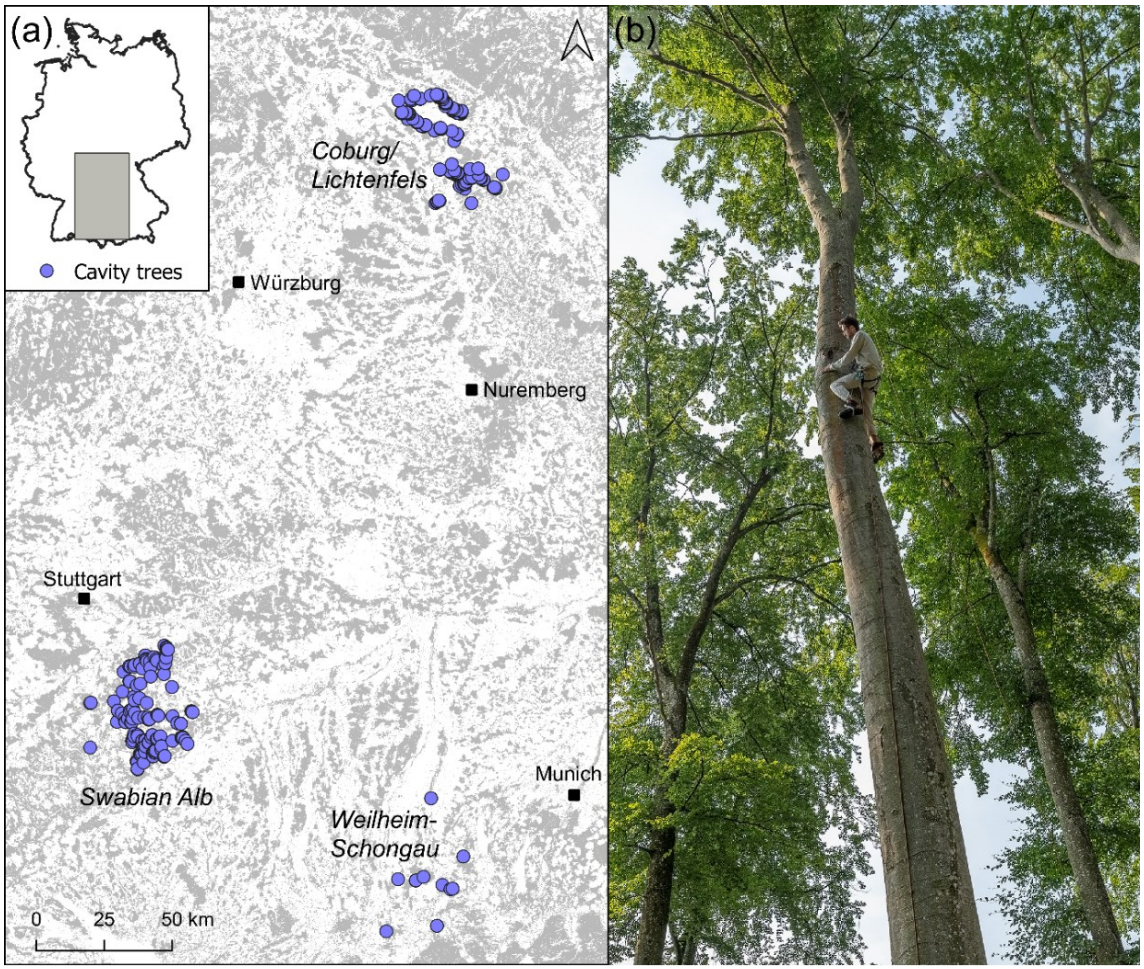

Figure 2.1: (a) Map of the cavity trees (blue dots) surveyed in three study regions in southern Germany. Forest areas are highlighted in grey (data from Weigand et al., 2020) and the locations of four cities are indicated by black squares as reference points. **(b)** Photo of a typical black woodpecker cavity tree in the Swabian Alb, with one of the authors (BR) inspecting the nest entrance of a feral honeybee colony (note that cavities were inspected from the ground during standard inspections). Photo by Ingo Arndt.

In the Swabian Alb and in Coburg/Lichtenfels we considered lists of 197 and 250 trees, respectively, which we inspected at least once during our study (see supplementary information for details on the selection of trees). The monitoring of many cavity trees in these two study regions allowed us to quantify cavity tree occupation rates and to estimate feral colony densities. In the third study region (Weilheim-Schongau), monitoring a large random sample of trees was not possible due to time constraints. There, we specifically surveyed 14 cavity trees which were known to have been used by honeybees (K. Zeimentz, personal communication). The observations from the third region were included in calculating feral colony survival rates. By collecting tree occupation and colony survival data from three distinct regions, we expected to

obtain information that would adequately represent the status of feral honeybee populations in comparable settings (managed forests) across Germany and beyond.

We inspected cavity trees three times per year in accordance with the honeybees' annual cycle of colony foundation, overwintering and reproduction. In July, after the main swarming season (in Germany: May and June; Henneken, Helm & Menzel, 2012; personal observations) we checked a high number of cavity trees to record the annual peak occupation rates. Between mid- and late September we re-inspected those cavity trees which had been previously occupied to determine the late summer survival, which might be critical due to the scarcity of floral resources (Garbuzov et al., 2020). Since new occupations were not to be expected between July and September, other trees were only inspected if they had not been checked before. From early to mid-April (before the swarming season), we determined the winter survival of all known honeybee colonies. Again, other trees were only checked if they had not been visited before. At all censuses, cavities were inspected with binoculars from the ground. We scored a cavity as being occupied if we observed bees that entered it and carried pollen (indicative of brood rearing activity in the nest). However, since recognizing pollen loads was not always possible due to the height of the cavities, we also accepted regular and directional in- and outward flight traffic as a positive indicator. If we only saw individual bees performing erratic zig-zag flights around the entrance, which is typical for scout and robber bees, we did not consider the cavity as inhabited by a living colony. In the Swabian Alb, the population monitoring started in September 2017 (Kohl & Rutschmann, 2018), and in Weilheim-Schongau and Coburg/Lichtenfels, the surveys started in April and July 2019, respectively. We conducted the last systematic census in April 2021. Colonies alive at that date were re-inspected once more in July 2021. For the Swabian Alb and for Coburg/Lichtenfels, we estimated feral population densities for each sampling date based on the cavity occupation rates and the known densities of the inspected trees (see supplementary information for details).

Genotyping bees to assess colony continuity

We could directly infer the summer and winter survival rates of feral honeybee colonies from the observed proportion of colonies alive in autumn and after winter, respectively. However, when a cavity was occupied both before and after the swarming season, this did not necessarily prove that the original colony had survived the spring. This is because nest sites which become vacant through colony death in spring can be quickly re-occupied by new swarms. To estimate the rate at which we would incorrectly note spring survival when the original colonies had actually died, we analysed the genetic relatedness of bees from a random subset of the cavities that had been occupied both before and after the swarming season.

For four to 18 workers and/or drones per colony we determined the DNA fragment lengths of 12 microsatellites representing a subset of the markers proposed by Shaibi, Lattorff & Moritz (2008)

at the Institute of Human Genetics of the University of Würzburg using a capillary sequencer (see supplementary text and Table S2.1 for details). We inferred the genotypes of the colonies' queens with the aid of the programme "COLONY" (Jones & Wang, 2010) and calculated coefficients of relatedness (Wang, 2007) between pairs of queens inhabiting the same tree at different time points using the package "related" for R (Pew et al., 2015). In case the relatedness between queens was at least 0.25 (grandmother–granddaughter relationship), we considered that the colony was the same before and after the swarming season, and thus that it had survived the spring.

Estimating demographic parameters

We obtained the colonies' annual survival rate (s) by multiplying the observed summer, winter, and spring survival rates. Based on published data on the probability of reproduction and the average number of swarms produced in temperate-adapted European honeybee colonies, we assumed that the average natality rate (n) in our population would be two swarms per wild-living colony per year (range: zero to four; Winston, 1980; Lee & Winston, 1987; Gilley & Tarpy, 2005; Seeley, 2017; see supplementary information). Based on the annual survival and natality rates we calculated the net reproductive rate (R_0) (Lotka, 1925; Krebs, 2014), which describes how the population of wild-living colonies would change from year to year if no immigration of swarms from managed hives occurred:

$$R_0 = s + s * n$$

Here, a value of $R_0 \geq 1$ indicates that the population is self-sustaining or expanding, while a value of $R_0 < 1$ indicates that it is dependent upon immigration. Another clear indicator of the population status of the wild-living colonies is the number of swarms (daughter colonies) that each colony would need to produce annually for the population to be self-sustaining (D). This statistic is easily interpreted when compared to the assumed natality rate of two swarms per colony per year (modified after Oldroyd et al., 1997):

$$D = \frac{1 - s}{s}$$

Both statistics are based on the following assumptions:

1) The wild-living colonies we considered for determining the annual survival rate represented a random sample. This is reasonable, since there are very few other cavities available in German forests apart from the woodpecker cavities that we surveyed (see above).
2) Most swarms produced by the wild-living colonies were able to find a new nest site. This was likely the case since most cavities were still vacant after the swarming season.

3) Most of the newly founded colonies survived the first weeks between their swarming event in May or June and our summer survey in July, i.e., we kept track of most colonies that ever colonized the trees monitored during the study period.
4) Colonies were unlikely to migrate from one tree to another without colony fission (in which case we would have noted the "death" of a nest although the number of colonies in the population stayed constant). This assumption is legitimate because simple colony migration or absconding is very rare in temperate-adapted honeybees (Winston, Taylor & Otis, 1983).
5) Swarms produced by wild-living colonies stayed part of the wild-living population. This assumption is valid, since neither do beekeepers usually install bait hives, nor are they likely to find and directly capture swarms issued by wild-living colonies in forest areas.

The average lifespan (L) of wild-living colonies can be estimated by summing over a range of age classes the products of each age (in years) and the probability of dying at that age:

$$L = \sum_{A=0}^{10} [A + 0.5][s^A][1 - s]$$

with A being the colony age in number of completed years and s, as above, being the annual survival probability for all colonies. With the use of this formula, it is assumed that colonies die, on average, halfway through a year (e.g., colonies that died within their first year are assigned a lifespan of 0.5 years), and that the annual survival probability is constant regardless of colony age. The latter can be justified by the fact that colonies are regularly taken over by young queens (during swarming or natural queen supersedure), so that colony lifespan is not restricted by queen longevity. However, the nests of honeybees age over time since they do not renew their beeswax combs, and this has possibly negative effects on colony development (Berry & Delaplane, 2001; Abd Al-Fattah, Yehia Ibrahim & Ibrahim Haggag, 2021). As a solution to avoid overestimating lifespan, we arbitrarily restricted the above summations to a maximum age class of $A = 10$, expecting that few colonies will ever live more than 10 years. Demographic studies of wild honeybees in the northeastern USA (Seeley, 1978, 2017) and in southeast Australia (Oldroyd et al., 1997) found that newly founded colonies have a significantly lower survival probability than established colonies aged at least one year. In such a case, the formula needs to be adapted (see supplementary information).

Statistical analyses

In calculating summer, winter, and spring survival rates, we pooled all colony observations across years and study regions. Since observations were not equally distributed over time and space, this procedure would have led to a bias in the estimates if survival rates had differed strongly between years or regions. However, this was not the case, so we believe that the reported survival rates are

accurate. We used two-sided Fisher's exact tests (in the case of two samples) and χ^2-tests (in the case of more than two samples) to test for differences in the proportions of surviving colonies or for differences in the proportions of occupied trees. All statistical tests were performed in R version 4.0.5 (R Core Team, 2022). Figures were created using QGIS version 3.16.8 (QGIS Development Team, 2021) and with the R-package ggplot2 (Wickham, 2016).

Results

Cavity tree occupation and population dynamics of feral honeybee colonies

The monitoring of cavity trees in the Swabian Alb and in Coburg/Lichtenfels revealed a recurring temporal pattern of population fluctuations: feral colony numbers peaked in summer, decreased moderately until autumn, dropped massively during winter, and recovered during the swarming season in spring (Figure 2.2). In July, mean cavity occupation rates were 12.9% in the Swabian Alb (range: 12%–14.5%, three summers 2018–2020) and 8.2% in Coburg/Lichtenfels (6.7% in 2019 and 9.7% in 2020). In September, average occupation rates were 11.1% in the Swabian Alb (four autumns, 2017–2020) and 6.5% in Coburg/Lichtenfels (two autumns, 2019 and 2020). In April, occupation rates had dropped to average values of 2% in the Swabian Alb (range 0–3.1%, four springs, 2018–2021) and 0% in Coburg/Lichtenfels (two springs, 2020 and 2021). Translating occupation rates into population densities revealed mean values of 0.18, 0.16 and 0.03 colonies per km^2 for the Swabian Alb and 0.30, 0.24 and 0 colonies per km^2 for Coburg and Lichtenfels for July, September, and April, respectively (Figure 2.2b). When averaging across years and study regions, the expected summer occupation rate and the respective maximum population density were 11% and 0.23 colonies per km^2, respectively. Accordingly, the minimum occupation rate and population density (after winter) were around 1.4% and 0.02 colonies per km^2.

Not only did the seasonal changes in tree occupation rates follow a predictable pattern, the spatial distribution of feral colonies did too: trees that had been occupied in the previous year but had become vacant during the winter, were five to 15 times more likely to be recolonized by new swarms than trees without recent bee occupation. This was revealed when considering cavity (re-)occupations in years in which all cavities had become free of bees during winter. Regarding the Swabian Alb, of the 11 trees which housed bees in summer 2018, 63.6% (seven trees) were re-colonized in 2019, while of the 79 trees that were not occupied in 2018 but reinspected in 2019, only 13.9% (11 trees) were colonized. In Coburg and Lichtenfels, 64.2% (nine out of 14) of the trees that were occupied in 2019 were re-colonized in 2020, while only 4.1% (seven out of 169) of the trees that were not occupied in 2019 were colonized in 2020. These differences in

occupation probabilities of former bee trees and non-bee trees were highly significant (P<0.001 for both Swabian Alb and Coburg/Lichtenfels, Fisher's exact tests).

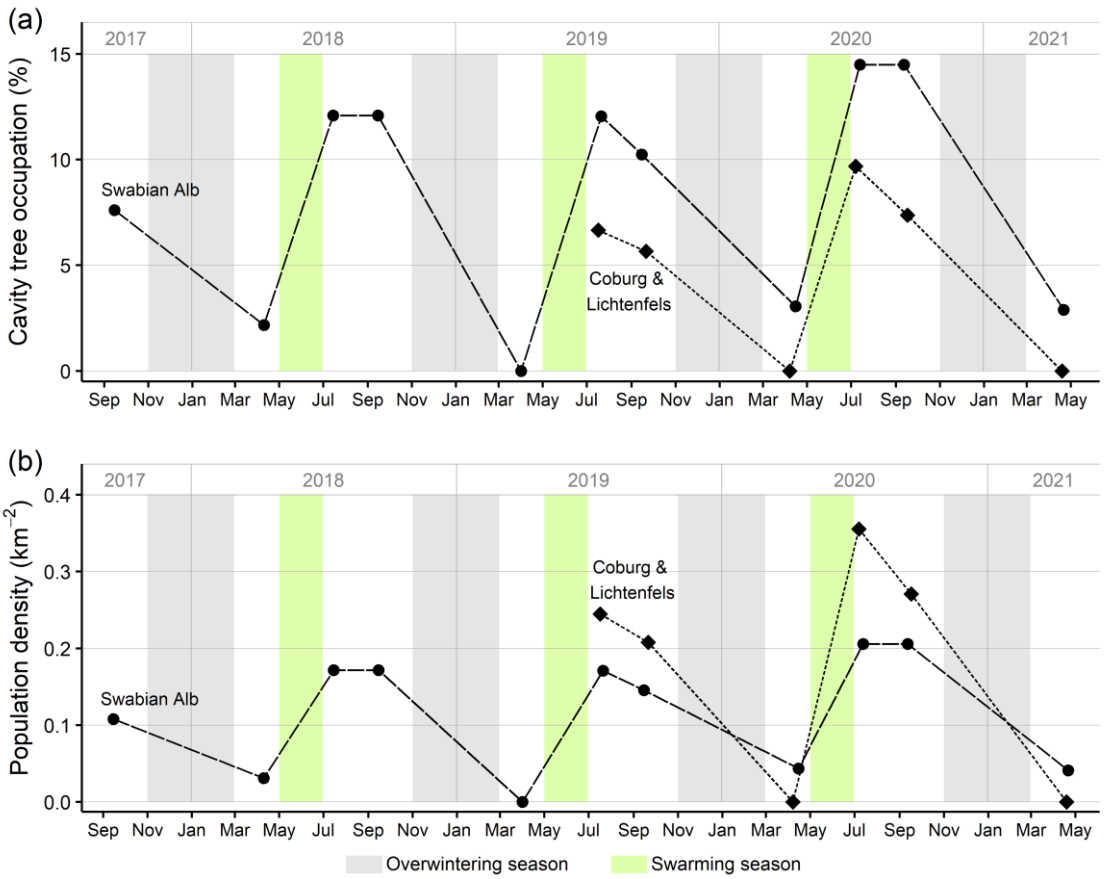

Figure 2.2: Temporal population fluctuations of feral honeybee colonies in forests of the Swabian Alb (September 2017–April 2021) and in the counties Coburg and Lichtenfels (July 2019–April 2021). The first data point (Swabian Alb, September 2017) has previously been reported (Kohl & Rutschmann, 2018). **(a)** Percentage of cavity trees occupied by feral honeybee colonies. See Table S2.2 for an overview of the numbers of colonies and trees considered. **(b)** Minimum population density of feral honeybee colonies inferred from cavity occupation rates and the known densities of cavity trees.

Population demography of feral honeybee colonies

We gathered data on feral colony survival from a total of N = 112 individual colonies from three woodland regions. While 90% of feral colonies survived the summer (July–September; N = 100 observations), only 16% survived the winter (September–April; N = 81). Considering spring survival (April–July), we found a total of 23 trees to be colonized in early spring, of which 19 (82.6%) were still occupied in summer ("apparent" spring survival). In nine of these 19 cases, we were able to genotype bees sampled from the colonies before and after the swarming season to determine the relatedness of their mother queens. In eight out of the nine cases (88.9%), the queens were closely related (at least mother-daughter relationships, see supplementary Tables S2.7.1–8), indicating that the respective trees had been continuously colonized by the same

colonies. We therefore estimate that the actual spring survival rate was 0.826 * 0.889 = 0.724 or 72.4%. The summer, winter and spring survival rates did not differ significantly between regions, nor between years (P>0.05, χ^2-tests, see Tables S2.3–5), nor between founder colonies (aged less than one year) and established colonies (aged at least one year) (P>0.3, Fisher's exact tests, see Table S2.6).

The annual survival rate of feral colonies resulting from the product of summer, winter and spring survival rates is s = 0.106 or 10.6%. Consequently, each colony would need to produce an average of D = 8.43 swarms annually to maintain the population. This clearly exceeds the assumed natality rate of n = two swarms produced per colony per year. Accordingly, the net reproductive rate of the feral honeybee population is R_0 = 0.318, indicating that it is currently not self-sustaining. The estimated average lifespan of feral colonies in German forests is 0.619 years.

Discussion

Despite the potential relevance of wild-living honeybee colonies in complementing managed colonies, until now, detailed studies on their population dynamics have been lacking in Europe. We conducted a demographic study to clarify the population status of feral honeybees in Germany. Our results show that feral honeybee colonies populate forests at densities of about one colony in 4–5 km^2 each summer, but that they do not form self-sustaining populations.

This conclusion is grounded on the result that only about one out of 10 feral colonies survived annually, meaning that successful colonies would need to produce eight to nine daughter colonies each swarming season for the population to be stable on its own. However, since temperate-adapted honeybee colonies only produce two swarms on average per year, we infer that the feral population would decrease if there was no immigration of foreign swarms. That immigration is indeed occurring every spring is evident, because the summer population densities of feral colonies varied little throughout the years of our study. In quantitative terms, we estimate that each year, around 70% of the forest-dwelling feral population must be recent immigrants (derived from the complement of its net reproductive rate, $1 - R_0$). The most likely source of these immigrants is the population of colonies managed by beekeepers in apiaries.

In recent years, several studies have reported on the occurrence of wild-living honeybee colonies in Europe (Oleksa, Gawroński & Tofilski, 2013; Kohl & Rutschmann, 2018; Requier et al., 2020; Browne et al., 2021; Dubaić et al., 2021; Moro et al., 2021; Oberreiter et al., 2021; Rutschmann et al., 2022). In the few cases where colony densities were estimated, the numbers were comparable to those reported here (rural avenues in Poland [Oleksa, Gawroński & Tofilski, 2013]: 0.1 colonies per km^2; Hainich National Park, Germany [Kohl & Rutschmann, 2018]: 0.13 colonies per km^2; agricultural landscape in NW Spain [Rutschmann et al., 2022]: 0.17–0.22 colonies per

km^2). In turn, all known wild-living populations which are evidently self-sustaining exhibit significantly higher colony densities: at least around one colony per km^2 in temperate regions (Seeley, 2019), and often greater than five colonies per km^2 in (sub)tropical regions (Jaffé et al., 2010; Rangel et al., 2016; Cunningham et al., 2022). Therefore, it seems likely that in many of the European cases wild-living colonies might merely represent recent escapees from apiaries. Importantly, our observations confirm the known habit of honeybee swarms to prefer cavities that have been used by bees before (Visscher, Morse & Seeley, 1985), meaning that reports about cavities that house wild honeybees for multiple years do not necessarily demonstrate that individual colonies live that long (Browne et al., 2021; Dubaić et al., 2021). For example, a recent study from Ireland (Browne et al., 2021) suggests that wild-living colonies commonly survive for 2–3 years, which would be indicative of a viable population (see Table 2.1). Unfortunately, it is unclear whether the reported survival times refer to colony lifespans or to the number of consecutive years a nest site was inhabited. Without robust estimates of colony survival rates the status of a given population of wild-living colonies remains ambiguous.

Two other populations of wild-living European honeybees, from the Arnot forest in the northeastern USA (Seeley, 1978, 2017, 2019) and from the Wyperfield National Park in southeast Australia (Oldroyd et al., 1997), have been investigated with respect to their demography (Table 2.1). The colony survival rates in these populations are around five times higher than in the German population (average survivorship >50% versus 11%), which is enough for them to be self-sustaining or even expanding. Furthermore, in the USA and Australia, colonies older than one year (established colonies) have a significantly higher annual survival probability than colonies younger than one year (founder colonies). This can be explained by the extra amount of energy that is needed for the foundation of a new nest, which involves building beeswax comb and food reserves from scratch (Seeley, 1978). In German forests, in turn, feral colonies had low survival rates regardless of their age. This suggests that either food availability is so low that not even established colonies can acquire enough, or that other factors are limiting colony survival. Regarding forage availability, the forests we worked at probably do not offer many nectar and pollen sources, since they are dominated by single wind-pollinated tree species (beech or spruce). In contrast, the deciduous forests in the northeastern USA usually contain several insect-pollinated tree species (e.g., *Acer* spp., *Tilia americana*) (Seeley, 2019) and the *Eucalyptus* woodlands in southeast Australia produce abundant nectar and pollen (Oldroyd et al., 1997). Furthermore, tree cavity densities are higher in Australian and North American forests compared to European forests (Remm & Lõhmus, 2011), so that forest-dwelling honeybees probably have more (and more diverse) nesting opportunities. Although we found that ca. 90% of the existing black woodpecker cavities were still vacant each summer, suggesting that the cavity density *per se* is not limiting, perhaps the cavities themselves are not optimal for honeybees. For example,

they might be too small to hold sufficient food stores for the winter, or too difficult to defend and thermoregulate given the relatively large entrance holes (Seeley & Morse, 1978). The fact that feral swarms preferentially occupied certain "bee trees" indeed suggests that many of the inspected black woodpecker cavities were not even attractive to the bees in the first place. Besides these ecological factors, the three investigated populations differ by an evolutionary factor. Both in the Arnot Forest and at Wyperfield National Park, wild-living colonies outnumber managed ones (Oldroyd et al., 1997; Seeley et al., 2015). Therefore, their populations can adapt evolutionarily to a life in the wild. In Germany, in contrast, the density of feral colonies is much lower than the density of managed ones (see below), so that the regional honeybee population is mainly shaped by the selection pressures prevailing under beekeeping management (Panziera et al., 2022). Today's most obvious selection pressure for wild-living honeybee populations, which is attenuated in apiculture, is the infestation by the parasitic mite *Varroa destructor* (Neumann & Blacquière, 2017). Indeed, there is evidence that wild honeybee populations from the northeastern USA differ genetically from sympatric managed populations and these differences are likely to involve adaptations which balance their relationship with *Varroa destructor* and its associated pathogens (Seeley et al., 2015; Mikheyev et al., 2016; Uribe et al., 2017). However, the feral colonies living in German forests have only recently left the apiary, meaning that they are unlikely to be genetically distinguishable from managed colonies and equally unequipped against the parasite.

Although feral honeybees in German forests are unlikely to bear genetic adaptations to parasites, they might still be relevant with respect to their effects on ecosystems. We found average population densities of 0.23 colonies per km^2 from early summer onwards. This number exceeds our previous estimate of 0.11 feral colonies per km^2 for managed beech forests in the Swabian Alb (Kohl & Rutschmann, 2018) because the latter was based on a census made in September 2017, when a fraction of that year's population had probably already died, and because the feral population density was generally lower in the Swabian Alb (0.18 colonies per km^2 in summer) than in our second reference region, Coburg/Lichtenfels (0.30 colonies per km^2). Under the assumption that our estimate of one colony in 4–5 km^2 approximately represents the feral colony density across the wider countryside in southern Germany, and considering that the average density of managed honeybees is around four colonies per km^2 (Baden-Württemberg: 5.21 colonies per km^2, Bavaria: 2.85 colonies per km^2; Deutscher Imkerbund, 2020), then feral colonies make up about 5% of the total honeybee population on a country-wide scale. However, after winter, when the feral populations have dropped to densities of only about one colony in 50 km^2, their share is much smaller (around 0.5%). At the local scale, the population density of feral honeybees will depend on the availability of cavities and the number of managed colonies within the dispersal range of swarms. For example, feral colonies should be relatively rare in intensive

agricultural areas due to the scarcity of nest sites, unless rural avenues are lined with hollow trees (Oleksa, Gawroński & Tofilski, 2013). Therefore, we can expect that swarms issued by managed hives in farmland typically disperse into nearby forests, if available. Indeed, the feral colonies we surveyed in our study must have almost exclusively stemmed from managed colonies in adjacent crops, grasslands, orchards, or villages. In cities, in turn, swarms escaping from managed hives are likely to find many nesting opportunities, whether it be cavities in old-grown trees in parks or hollow spaces in man-made structures (Browne et al., 2021; Dubaić et al., 2021), so that managed and feral colonies will live spatially intertwined.

Table 2.1: Demographic parameters of three populations of wild-living honeybee colonies. Information is provided on the location of the populations, the annual survival rates of colonies (either for all colonies, s, or for founder and established colonies separately, f and e), the average lifespan of wild-living colonies (L, in years), the number of swarms needed to be produced per colony and year for the population to be self-sustaining (D), and the net reproductive rate of the populations (R_0).

Population	**s**		**L** [$]	**D** *	**R_0** *	**Reference**
	(f)	**[e]**				
Arnot Forest, USA	(0.24)	[0.79]	1.34	0.94	1.55	(Seeley, 1978, 2017)[#]
Wyperfield NP, Australia	(0.32)	[0.76]	1.53	0.85	1.62	(Oldroyd et al., 1997)
German forests	0.11		0.62	8.43	0.32	This study

[$] The average colony lifespan of the Arnot forest and Wyperfield populations deviate from what was reported in the original studies since we used a modified calculation (see supplementary information).
*To calculate D and R in the case of the Arnot forest and the Wyperfield populations, we considered as the annual survivorship of all colonies (s) the mean of the survival rates of founders (f) and established colonies (e).
[#] Seeley (Seeley, 2017) presents in the Appendix 1 of his paper an overview of the number of colonies that survived and died during his population studies in the 1970s and in the 2010s. He distinguished between summer and winter survival and between founder and established colonies. We used these data to calculate average annual survival rates for founder and established colonies.

Our study showcases that the feralisation (Daniels & Bekoff, 1989) of honeybees is much more common than previously assumed. Germany-wide, tens of thousands of swarms will emigrate from apiaries each spring to found feral colonies in tree holes or other cavities. Therefore, it is imprecise to consider the honeybee population as fully managed or domesticated, and it needs to be recognized that the impact of beekeeping on the environment goes beyond the effect of bees foraging in the area around apiaries. Whether beekeepers' incidental "service" of issuing feral swarms to the surroundings is generally beneficial or not is currently unclear (Saunders et al., 2021). Questions remain about whether low versus zero abundances of feral honeybees affect the pollination of wild plants in forests (Hung et al., 2018), how honeybees interact with other organisms in tree cavities (Reinsch, 1979; Paton, 1996; Sikora, Schnitt & Kinser, 2016), and

whether feral honeybees play a role in the transmission of parasites and pathogens to managed honeybees and non-*Apis* bees (Frey & Rosenkranz, 2014; Fürst et al., 2014; Thompson et al., 2014; Youngsteadt et al., 2015; Mallinger, Gaines-Day & Gratton, 2017; Tehel et al., 2022). Furthermore, with the goal to improve the wellbeing of all honeybees, it is important to know why feral colonies currently fail to establish self-sustaining populations, whether it be due to ecological (e.g., lack of floral food resources and suitable nesting sites, parasite pressure) or evolutionary factors (domestication).

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Supplementary information

Selection of cavity trees in the Swabian Alb and in Coburg & Lichtenfels

From the original lists of cavity trees in the Swabian Alb (Sikora, Schnitt & Kinser, 2016) and in Coburg & Lichtenfels (N. Wimmer, personal communications) we first shortlisted samples that were preferably located in coherent forest areas well accessible via forest roads. Trees which could not be found, whose cavity entrances were not visible from the ground, or which we considered unsuitable as nest sites for honeybees upon our first inspections (e.g., lying and standing dead wood, completely hollow trees) were excluded from the lists. In case we discovered new cavity trees, or when other trees occupied by honeybees were reported to us during the study period, these were also considered for the survey (approx. 10% of all trees). This resulted in lists of 197 and 250 trees for the Swabian Alb and Coburg/Lichtenfels respectively, which we

inspected at least once during our study. Note that not all trees were monitored over the whole study period.

Estimating feral population densities from known tree densities and occupation rates

In the Swabian Alb, the overall black woodpecker cavity tree density was reported to be 1.6 trees per km^2 (Sikora, 2009), but we deemed only 88.7% of the trees as appropriate for honeybees (see above). This led to a cavity tree density of 1.42 trees per km^2 as the baseline. In Coburg and Lichtenfels, the cavity tree density was 4.4 trees per km^2 (N. Wimmer, personal communication) and we considered 83.5% of the inspected trees in our surveys, leading to a baseline cavity tree density of 3.67 trees per km^2. In calculating cavity tree occupation rates and feral population densities we only considered cavity trees that we had inspected during our systematic censuses, i.e., that represented a random sample. Before the winters 2019/20 and 2020/21, approximately half of the cavity trees were manipulated by us as part of another study. Since the treatments might have influenced the colonies' winter survival, we did not consider the manipulated colonies for estimating the overall winter survival rate, nor did we use the respective trees for estimating occupation rates and population densities for the following springs. For example, if we observed 10 feral colonies in 100 trees and manipulated 50% of these colonies (five colonies) in autumn, we would only consider the winter survival of the other five (non-manipulated) colonies. Accordingly, for determining the tree occupation rate (and the feral population density) in the following spring, we would only consider the number of survivors among the five non-manipulated colonies and consider 50 trees (100 trees*0.5) as the denominator.

Calculating the average lifespan of feral honeybee colonies

The formula for calculating average lifespan presented in the main text assumes that the annual survival rate (s) is constant regardless of colony age. This assumption might be incorrect, as indicated by studies of the population demography of wild-living honeybees in the northeastern USA (Seeley, 1978, 2017) and in southeast Australia (Oldroyd et al., 1997), respectively. In both populations, newly founded colonies have a significantly lower survival probability than colonies aged at least one year. In such a case, the formula needs to be adapted as follows (modified after Seeley, 1978):

$$L = 0.5[1 - f] + \sum_{A=1}^{10} [A + 0.5][(f)(e)^{A-1}][1 - e]$$

Here, f is the probability of first-year survival (founders) and e is the probability of annual survival for all subsequent years (established colonies). Again, the summation is restricted to a maximum age class of $A = 10$, since honeybee colonies are unlikely to live longer than 10 years.

Microsatellite genetic analyses

Sampling bees from feral colonies

To collect bees from nests in tree cavities, we used a white insect net mounted to an approx. 10-m telescopic rod. If the cavity entrance was too high, we ascended the first few metres using a climbing technique inspired by traditional tree beekeeping methods (taught to us by Luis G. Sikora): Three semi-static climbing ropes, two equipped with food straps and one connected to a chest strap, were looped around the trunk. The "lassos" secured the climbing person and were used to directly walk up the tree (Figure S2.1a). For sampling, we kept the insect net stretched out using a metal bow and we narrowed the opening to the approximate size of the cavity entrance using black nylon stocking. This modified net effectively caught bees flying out of the nest when positioned in front of the cavity entrance (Figure S2.1b). The sampled bees were directly killed with ethyl acetate and stored in 99% ethanol.

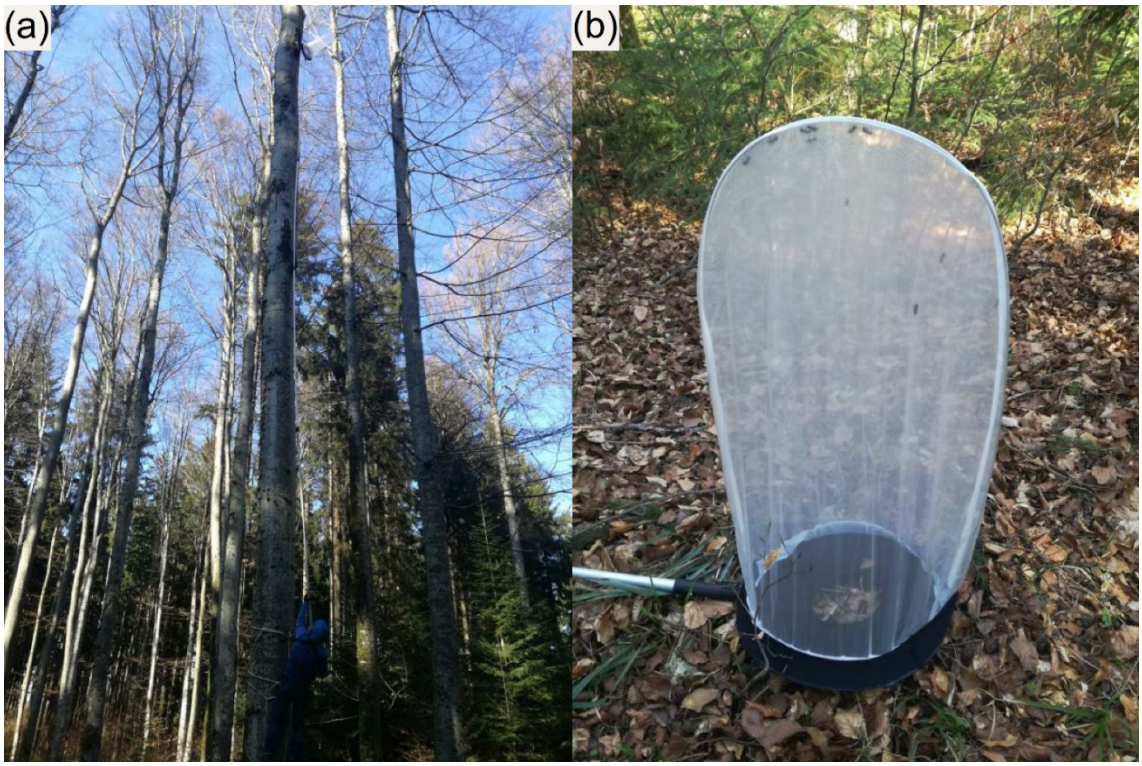

Figure S2.1: The method used to collect bees from feral colonies. **(a)** The cavity entrance of a bee tree was reached with an insect net by combining a "lasso" climbing technique and a telescopic rod. **(b)** The modified insect net trapped bees flying out of the nest.

DNA extraction

We removed both hindlegs of each bee with clean scissors, cut them into pieces and extracted their DNA using the "NucleoSpin® Tissue XS" kit by Macherey-Nagel (Düren, Germany; www.mn-net.com) according to the manufacturers protocol (in steps 2, 3 and 4 we used 120 µL of Buffer T1, Buffer B3 and ethanol, respectively). This yielded 20 µL of extract per bee, with an

average DNA concentration of 12.7 ng/µL (range: 2.4–35.7 ng/µL, concentration of N = 17 representative samples measured using a Qubit® 2.0 fluorometer)

PCR and fragment length analyses

We had initially planned to genotype our bees at the full set of 18 microsatellite DNA markers proposed by Shaibi, Lattorff & Moritz (2008). The toolkit consists of nine marker groups: six unlinked loci, and three groups each encompassing four tightly linked loci. The markers can be amplified in two multiplex PCR reactions (each with nine markers distinguishable by fragment lengths and fluorescent labels). We used the dye "ATTO 550" instead of "TET" as one of the three types of fluorescent labels for the primers, so that we could analyse the markers with Applied Biosystems' filter set "D" in an ABI 3130xl capillary sequencer. Otherwise, we sticked to the original protocol (Shaibi, Lattorff & Moritz, 2008). However, in our first tests, only nine markers were amplified satisfactorily (loci that did not work: HB-SEX-01, HB-SEX-02, HB-SEX-03, UN351, HB-THE-01, HB-THE-02, HB-THE-03, HB-C16-01, HB-C16-02). In the following we present a modified protocol which allowed us to analyse 12 of the 18 loci, including all six unliked loci and two groups of linked loci (see Table S2.1). The modifications involved splitting the two multiplex PCR reactions into four (1A, 1B, 2A, 2B), implementing three temperature steps for primer annealing in each reaction cycle (to assure that also primers with extreme annealing temperatures would bind to the DNA), and reducing the primer concentration of loci that had been disproportionally amplified (since these might have competed too much with the primers of poorly amplified loci). We do not know in which ways these modifications improved PCR results. We also did not perform exhaustive testing, so there might have been better solutions that would have enabled analysing more than the 12 loci. However, since 12 loci were enough to discriminate related from unrelated bees, the presented protocol served the purpose.

- The PCR solutions contained 5 µL of Promega Master Mix, 2 µL of (multi-)primer solution (concentration: 1 or 0.5 µM of each primer in the primer solution and hence 0.2 or 0.1 µM per primer in the final PCR solution, see Table S2.1), 2 µL of nuclease-free water, and 1 µL of DNA extract (see above).
- The PCRs were run in a "SureCycler 8800" (Agilent Technologies) with the following programme:
  - 5 min of initial denaturation at 95 °C,
  - 35 cycles of: 30 s of denaturation at 95 °C, 5 s of annealing at 65 °C, 15 s of annealing at 55 °C, 30 s of annealing at 48 °C, 1 min of elongation at 72 °C,
  - 20 min final elongation at 72 °C.
- After PCR, we mixed the products of reactions 1A and 1B and of reactions 2A and 2B to obtain two multiplex mixtures per sample, according to the original protocol by Shaibi, Lattorff & Moritz (2008).

- To prepare for fragment length analyses, we added 1 µL of the mixed PCR products to 20 µL Formamide and 0.5 µL "ROX"-labelled DNA size standard (ILS 600, Promega), and denatured the fragments by placing the solutions into a thermocycler for 1 min at 94°C.

Table S2.1: Microsatellite markers used in this study with information on their distribution over two (four) multiplex PCRs, the observed fragment size ranges, the size of the sequence repeat motifs used by the programme TANDEM to bin raw allele length into integer allele size classes (see below), the number of observed alleles and the number of observed haplotypes (in the case of linked marker groups). The concentration of the primers of locus HB-THE-02 were reduced to 0.1 µM in the final reaction (all other primers: 0.2 µM).

Locus	Plex (Shaibi et al. 2008)	Plex (this study)	Min. allele size (bp)*	Max. allele size (bp)*	Repeat size (bp)	Number of Alleles	Number of Haplotypes
A079	2	2A	89	115	2	11	-
AP043	2	2A	132	158	2	7	-
A113	2	2A	198	228	5	6	-
A024	1	1A	95	105	2	4	-
A107	1	1A	149	177	2	15	-
A007	1	1A	97	121	2	10	-
HB-THE-02	2	2B	235	245	2	6	14
HB-THE-04	2	2A	225	231	2	4	
HB-C16-01	2	2B	248	310	2	19	108
AC006	1	1A	148	163	3	5	
HB-C16-02	1	1B	235	297	2	26	
HB-C16-05	1	1A	68	102	2	14	

*Allele sizes ranges might not be accurate since we did not sequence individual fragments (e.g., the minimum and maximum allele sizes of locus A079 might well be 90 bp and 116 bp instead of 89 and 115 bp).

Generating tables with the genotypes of the sampled bees

We manually determined microsatellite fragment lengths from electropherograms (produced by the capillary sequencing machine, FSA-files) using the programme "Fragman" (Covarrubias-Pazaran et al., 2016) for R (R Core Team, 2022). Various factors influence the migration rate of DNA fragments in capillaries during electrophoresis ("allelic drift"), so that observed allele sizes (based on their migration rate relative to the size standard) are usually not completely accurate and do not conform to whole base pair numbers (e.g., the measured length of an allele might be "101.53" bp, leaving the researcher unsure about whether the actual allele size is 101 or 102 bp) (Guichoux et al., 2011). Simply rounding to the next integer does not solve the problem, since not all fragment sizes are equally likely to occur depending on the repeat motif of the microsatellite sequence (Guichoux et al., 2011). To account for allelic drift and the sequence repeat motifs of each marker, we translated raw allele lengths into integer allele sizes ("allele binning") using the software "TANDEM" (Matschiner & Salzburger, 2009).

The 12 analysed microsatellites were composed of six unlinked markers and two groups of tightly linked markers, i.e., microsatellites that are located in close proximity on the same chromosome and which are hence unlikely to be separated during chromosomal crossover. The linked loci are useful for the discrimination of related individuals due to the high number of potential variants (haplotypes) that arise when the alleles of several linked markers are considered together (Shaibi, Lattorff & Moritz, 2008). To determine which alleles of the linked loci occur together on each of the two homologous chromosomes in diploid (worker) samples, we used the programme "PHASE" (Stephens, Smith & Donnelly, 2001). The linked-loci haplotypes were subsequently treated like single-locus alleles.

Inferring colony turnover versus continuity

We used the programme COLONY (Jones & Wang, 2010) to infer the genotypes of the colonies' queens based on the observed genotypes of sampled workers and drones. We then computed Wang's (2007) coefficient of relatedness for pairs of queens that inhabited the same nest before and after the swarming season (assuming the population-wide allele frequencies estimated by COLONY) using the programme "related" for R (Pew et al., 2015; R Core Team, 2022). We considered a cavity as being occupied by the same colony before and after the swarming season if the relatedness of queens was at least 0.25. For example, two queens could be found to be the same (relatedness: 1) before and after spring when a colony survived but neither swarmed nor superseded its queen. One hundred % relatedness of inferred queens could also arise when the colony *did* swarm, but the offspring of the new queen were not old enough to frequently leave the nest and thus unlikely to be sampled by us (it takes about eight weeks after swarming until a new queen's offspring enters the population of foraging bees of a colony). Queens would be related by 50% when the colony swarmed and was then naturally taken over by one of her daughters. Lastly, queens of the same colony would be related by 25% (grandmother-granddaughter relationship), for example, when a colony first superseded its old queen and then entered a cycle of swarming. Note that samples were either taken in April and July (right before and after the swarming season), or in July of the previous year and in July right after the swarming season under consideration. Especially in the second case, the queens of a surviving colony could well have well been only 25% related.

The swarming rate of feral honeybee colonies

The natality rate in a population of wild-living honeybee colonies (the average number of swarms produced per colony per year) is difficult to determine, since it would require the continuous observation of the nest entrances of many colonies. We therefore relied on existing knowledge of the swarming rate of unmanaged colonies in man-made hives that resembled the situation of wild-living colonies. Observations of queen turnover rates of wild-living honeybee colonies from the

Arnot forest in the north-eastern USA suggest that 87% of colonies entering the swarming season will reproduce (Seeley 2017). We further know from three studies reporting the swarming rate of unmanaged colonies that they cast 2.3 swarms on average (range: one to four): Winston (1980) reported an average swarming rate of three swarms per colony during the main (spring) swarming season based on the observation of five colonies in Kansas (one of the five observation colonies swarmed again later in the year, but we neglect this secondary swarming phase here since in Germany swarming happens mostly in spring). Lee and Winston (1987) reported a natality rate of 2.2 swarms per colony per year based on the observation of 14 colonies over two years in British Columbia. Gilley & Tarpy, (2005) reported on the fate of young queens after the departure of primary swarms in a total of six observation colonies in Ithaca (New York) and Scotland (citing Allen, 1957). These colonies produced a total of 10 swarms, which equals an average swarming rate of 1.667 swarms per colony. We therefore assumed that the average natality rate in our population would be two swarms per colony per year (0.87 * [3+2.2+1.667]/3 swarms). This rate is plausible for unmanaged honeybees in Germany (Zander & Weiss, 1964, personal observations).

Table S2.2: Overview of the number of feral colonies and the number of cavity trees considered at each survey round for calculating cavity tree occupation rates and feral population densities.

Study region	Survey month	Mean survey date	Number of colonies	Number of trees	Tree occupation (%)	Population density (colonies per km^2)
Swabian Alb	Sep 17	15.09.2017	7	92*	7.61*	0.11
	Apr 18	10.04.2018	2	92	2.17	0.03
	Jul 18	15.07.2018	11	91	12.09	0.17
	Sep 18	15.09.2018	11	91	12.09	0.17
	Apr 19	01.04.2019	0	159	0	0.00
	Jul 19	21.07.2019	20	166	12.05	0.17
	Sep 19	15.09.2019	17	166	10.24	0.15
	Apr 20	15.04.2020	3	98	3.06	0.04
	Jul 20	13.07.2020	20	138	14.49	0.21
	Sep 20	12.09.2020	20	138	14.49	0.21
	Apr 21	21.04.2021	2	69	2.90	0.04
Coburg & Lichtenfels	Jul 19	17.07.2019	11	165	6.67	0.24
	Sep 19	21.09.2019	12	212	5.66	0.21
	Apr 20	07.04.2020	0	141	0	0.00
	Jul 20	07.07.2020	21	217	9.68	0.36
	Sep 20	17.09.2020	16	217	7.37	0.27
	Apr 21	19.04.2021	0	81	0	0.00

*The results of the first tree census in September 2017 have been published before (Kohl & Rutschmann, 2018). In the original publication, it was indicated that 98 cavity trees, not 92, had been surveyed. Accordingly, the tree occupation rate was reported to be 7.1%, not 7.6%. Here, we consider only 92 reference trees for September 2017 because we sorted out a fraction of the original trees which, due to growing experience during our study, we gauged not to bear intact cavities. However, in estimating feral population densities, we accounted for not having considered all cavity trees (see above). Therefore, the density estimates are accurate and the estimate of 0.11 colonies per km^2 presented here matches the number reported earlier.

Table S2.3: Comparison of apparent spring (April–July), summer (July–September) and winter (September–April) survival rates of feral honeybee colonies between the three study regions. Numbers in brackets indicate the number of colonies that survived, and the number of colonies observed.

Study region	Spring survival	Summer survival	Winter survival
Swabian Alb	0.9 (9/10)	0.944 (51/54)	0.163 (7/43)
Coburg & Lichtenfels	0.75 (3/4)	0.829 (29/35)	0 (0/17)
Weilheim-Schongau	0.778 (7/9)	0.909 (10/11)	0.286 (6/21)
Differences between regions?	$\chi^2 = 0.688$ d.f. = 2 P = 0.709	$\chi^2 = 3.179$ d.f. = 2 P = 0.204	$\chi^2 = 5.696$ d.f. = 2 P = 0.058

Table S2.4: Comparison of apparent spring (April–July), summer (July–September) and winter (September–April) survival rates of feral honeybee colonies in different study years. Numbers in brackets indicate the number of colonies that survived and the number of colonies observed.

Study year	Spring survival	Summer survival	Winter survival
2017	–	–	0.286 (2/7)
2018	1 (2/2)	1 (11/11)	0 (0/13)
2019	0.667 (2/3)	0.879 (29/33)	0.258 (8/31)
2020	0.923 (12/13)	0.893 (50/56)	0.1 (3/30)
2021	0.6 (3/5)	–	–
Differences between years?	$\chi^2 = 3.582$ d.f. = 3 P = 0.310	$\chi^2 = 1.419$ d.f. = 2 P = 0.492	$\chi^2 = 6.305$ d.f. = 3 P = 0.098

Table S2.5: Chronological overview of apparent spring (April–July), summer (July–September) and winter (September–April) survival rates for each study region.

Region	Year	Spring survival	Summer survival	Winter survival
Swabian Alb	2017	–	–	0.286 (2/7)
	2018	1 (2/2)	1 (11/11)	0 (0/13)
	2019	–	0.864 (19/22)	0.25 (3/12)
	2020	0.8 (4/5)	1 (21/21)	0.182 (2/11)
	2021	1 (3/3)	–	–
Coburg & Lichtenfels	2019	–	0.909 (10/11)	0 (0/8)
	2020	1 (3/3)	0.792 (19/24)	0 (0/9)
	2021	0 (0/1)	–	–
Weilheim-Schongau	2019	0.667 (2/3)	–*	0.455 (5/11)*
	2020	1 (5/5)	0.909 (10/11)	0.1 (1/10)
	2021	0 (0/1)	–	–

*We have no data for 2019 summer survival (period July–September) from Weilheim-Schongau since the 11 colonies found there in July were not re-inspected in autumn 2019 due to time constraints. In spring 2020, five of the 11 colonies were still alive, but it is unclear how many colonies died in the summer and in the winter. For practical reasons, we assumed that all 11 colonies had survived the summer period (which is realistic based on what we observed in other years/regions) and counted the 11 cases as observations of winter survival.

Table S2.6: Comparison of apparent spring (April–July), summer (July–September) and winter (September–April) survival rates between colonies aged less than one year (founders) and colonies older than one year (established colonies). The overall survival rates are also given ("all colonies"). We did not know the history of all colonies so that the total number of observations exceeds the sum of founder and established colony observations. *P*-values are results of Fisher's exact tests.

Colony type	Spring survival	Summer survival	Winter survival
All colonies	0.826 (19/23)	0.90 (90/100)	0.160 (13/81)
Founder colonies	0.75 (6/8)	0.910 (61/67)	0.103 (4/39)
Established colonies	0.667 (2/3)	0.857 (12/14)	0.214 (3/14)
Difference founder vs. established?	$P = 1.00$	$P = 0.622$	$P = 0.364$

Table S2.7.1–8: Observed genotypes of workers (w) and drones (d) sampled from feral honey bee colonies before and after the swarming season, and the inferred genotypes of their mother queens (q). The alleles at six unlinked and at two groups of linked microsatellite loci are presented as relative numbers of sequence repeats, e.g., allele "1" at locus AP043 stands for a fragment of 132 bp (which is the smallest fragment found for this locus) and allele "5" stands for a fragment of 140 base pairs (see Table S2.1). Sample IDs encode the study region (S = Weilheim-Schongau, A = Swabian Alb, C = Coburg and Lichtenfels), the tree ID within each study region, and the time point of sampling, e.g., S-16-19a was the first of two samples from a colony nesting in tree number 16 in Weilheim-Schongau taken in 2019 (exact sampling dates are given in brackets). Sampled bees (bee ID) marked with an asterisk have genotypes that do not match those of the colonies' queens at one of the eight loci/linkage groups (possible reasons: microsatellite scoring error/ individuals are daughters of the preceding queen/ individuals stem from foreign colony). NAs are given when alleles were absent or not scorable from electropherograms. Relatedness values report the parameter developed by Wang (2007).

Sample ID	Bee ID	Bee type	A079	AP043	A113	A024	A107	A007	HBTHE02 – HBTHE04	HBC1601 – AC006 – HBC1602 – HBC1605
S-16-19a (15.4.2019)	1	w	7 8	6 14	3 4	4 4	3 6	1 6	3 2 6 2	NA NA
	2	w	5 8	5 6	3 3	4 5	5 11	1 4	5 1 6 2	NA NA
	3	w	8 7	6 14	4 4	4 4	3 6	6 6	3 2 5 1	18 3 8 2 20 3 24 2
	4	w	7 8	6 6	1 4	4 5	6 11	1 8	4 2 5 1	NA NA
	5*	w	8 10	6 6	3 3	5 5	6 11	4 6	4 2 6 2	18 3 14 2 18 3 24 2
	6	w	4 8	6 7	3 4	4 5	6 9	1 9	4 2 5 1	20 3 24 2 25 3 25 3
	7*	w	1 8	1 6	3 4	4 4	6 11	1 3	4 2 6 2	20 3 10 2 32 3 10 2
	8	w	6 8	5 6	3 4	4 5	4 11	1 6	4 2 5 1	20 3 24 2 23 6 15 2
	9	w	4 8	6 7	3 4	4 5	9 11	1 9	4 2 5 1	18 4 29 2 25 3 25 3
	10	w	8 8	6 6	3 3	4 4	5 6	6 7	2 2 6 2	NA NA
	11	w	8 10	6 6	3 4	5 5	6 11	1 4	4 2 5 1	18 3 14 2 20 3 24 2
		q	**8 8**	**6 6**	**3 4**	**4 5**	**6 11**	**1 6**	**5 1 6 2**	**18 4 29 2 20 3 24 2**
S-16-19b (30.7.2019)	12	w	1 8	1 6	3 4	4 5	6 6	4 6	4 2 6 2	20 3 24 2 31 3 10 2
	13	w	8 9	6 6	3 3	5 5	11 11	1 4	4 2 5 1	NA NA
	14	w	5 8	5 6	3 3	4 4	5 6	1 4	NA NA	NA NA
	15	w	8 10	5 6	4 4	1 4	4 6	1 4	4 1 5 1	4 3 9 2 20 3 24 2
	16	w	6 8	1 6	4 4	5 5	3 6	5 6	6 2 6 2	20 3 17 2 20 3 24 2
	17	w	8 8	5 6	3 6	4 4	6 6	6 6	2 1 6 2	14 3 25 2 18 4 29 2
	18	w	8 10	6 6	3 4	5 5	11 11	4 6	4 2 5 1	18 3 14 2 18 4 29 2
	19	w	8 8	5 6	3 6	4 4	6 6	6 6	2 1 5 1	14 3 25 2 20 3 24 2
	20	w	8 8	6 6	4 4	4 5	3 6	1 6	3 2 5 1	18 3 8 2 18 4 29 2
	21*	w	8 10	5 6	3 4	4 5	3 6	1 6	4 1 5 1	4 3 8 2 20 4 29 2
	22	w	8 10	6 6	3 3	5 5	11 11	1 4	4 2 5 1	18 3 14 2 20 3 24 2
	23	w	7 8	6 6	3 4	4 5	3 6	6 6	3 2 5 1	18 3 8 2 20 3 24 2
	24	w	NA NA	NA NA	NA NA	5 5	11 11	4 6	NA NA	NA NA
		q	**8 8**	**6 6**	**3 4**	**4 5**	**6 11**	**1 6**	**5 1 6 2**	**18 4 29 2 20 3 24 2**

Estimated relatedness of queens: 1

Table S2.7.2

Sample ID	Bee ID	Bee type	A079	AP043	A113	A024	A107	A007	HBTHE02 – HBTHE04	HBC1601 – AC006 – HBC1602 – HBC1605
S-5-19a (16.4.2019)	25	w	6 8	5 6	4 7	4 4	7 11	6 7	4 2 5 2	1 6 28 2 21 3 12 12
	26	w	7 9	5 6	3 4	4 5	7 7	6 6	4 1 4 2	4 6 4 16 22 3 9 2
	27	w	7 8	5 6	3 4	4 4	3 10	6 7	4 1 5 2	1 6 28 2 21 3 12 12
	28	w	7 14	6 7	3 3	5 5	6 7	7 8	4 2 4 2	4 6 3 8 22 3 9 3
	29	w	6 9	1 6	3 7	4 4	3 4	6 7	4 1 5 2	1 6 28 2 4 7 3 2
	30	w	6 6	2 5	7 7	4 5	1 7	6 7	3 1 4 1	4 6 3 7 22 3 9 2
		q	**6 7**	**5 6**	**3 7**	**4 5**	**3 7**	**6 7**	**4 1 4 2**	**1 6 28 2 22 3 9 2**
S-5-19b (30.7.2019)	31	w	4 6	2 5	4 7	4 5	3 7	6 6	4 2 5 2	1 6 28 2 18 3 3 2
	32	w	7 9	1 5	3 7	4 4	4 7	6 7	4 2 5 2	1 6 28 2 4 7 3 2
	33	w	7 8	6 6	4 7	4 4	7 10	7 7	4 2 5 2	21 3 12 12 22 3 9 2
	34	w	4 7	2 5	4 7	4 5	7 7	6 6	4 1 4 2	18 3 22 2 22 3 9 2
	35	w	7 13	5 5	3 7	5 5	4 7	7 8	4 1 4 2	4 6 3 7 22 3 9 2
	36*	w	7 8	4 5	4 7	5 5	8 8	7 8	4 2 4 2	19 6 31 2 22 3 9 2
	37	w	6 8	4 6	3 4	4 5	7 8	7 7	4 1 4 2	20 6 32 2 22 3 9 2
	38	w	7 9	1 5	3 7	4 4	4 7	4 7	4 1 5 2	4 7 3 2 22 3 9 2
	39	w	6 9	2 5	4 7	4 5	7 9	6 7	4 2 4 2	1 6 28 2 18 3 23 2
	40	w	7 8	6 6	3 4	4 4	7 10	6 7	4 1 5 2	1 6 28 2 21 3 12 12
	41	w	6 8	4 6	4 7	4 5	7 8	6 7	4 1 4 2	1 6 28 2 20 6 32 2
	42	w	6 13	5 5	3 3	4 5	3 6	6 7	4 2 4 2	4 6 3 7 22 3 9 2
	43	w	7 14	5 5	3 7	4 5	3 6	7 7	4 1 4 2	1 6 28 2 4 6 3 7
	44	w	7 8	1 5	3 7	5 5	7 8	7 8	4 1 5 2	1 3 22 2 1 6 28 2
	45	w	6 9	1 6	3 3	4 4	3 4	7 7	4 1 5 2	1 6 28 2 4 7 3 2
	46	w	6 8	4 6	3 4	4 5	3 8	6 7	4 1 4 2	NA NA
	47	w	6 13	5 6	3 7	4 5	6 7	6 6	4 1 4 2	4 6 3 7 22 3 9 2
	48	w	7 13	5 5	3 3	4 5	6 7	6 7	4 2 4 2	1 6 28 2 4 6 3 7
		q	**6 7**	**5 6**	**3 7**	**4 5**	**3 7**	**6 7**	**4 1 4 2**	**1 6 28 2 22 3 9 2**

Estimated relatedness of queens: 1

Table S2.7.3

Sample ID	Bee ID	Bee type	A079	AP043	A113	A024	A107	A007	HBTHE02 – HBTHE04	HBC1601 – AC006 – HBC1602 – HBC1605
S-5-19b[#] (30.7.2019)	31	w	4 6	2 5	4 7	4 5	3 7	6 6	4 2 5 2	1 6 28 2 18 3 3 2
	32	w	7 9	1 5	3 7	4 4	4 7	6 7	4 2 5 2	1 6 28 2 4 7 3 2
	33	w	7 8	6 6	4 7	4 4	7 10	7 7	4 2 5 2	21 3 12 12 22 3 9 2
	34	w	4 7	2 5	4 7	4 5	7 7	6 6	4 1 4 2	18 3 22 2 22 3 9 2
	35	w	7 13	5 5	3 7	5 5	4 7	7 8	4 1 4 2	4 6 3 7 22 3 9 2
	36*	w	7 8	4 5	4 7	5 5	8 8	7 8	4 2 4 2	19 6 31 2 22 3 9 2
	37	w	6 8	4 6	3 4	4 5	7 8	7 7	4 1 4 2	20 6 32 2 22 3 9 2
	38	w	7 9	1 5	3 7	4 4	4 7	4 7	4 1 5 2	4 7 3 2 22 3 9 2
	39	w	6 9	2 5	4 7	4 5	7 9	6 7	4 2 4 2	1 6 28 2 18 3 23 2
	40	w	7 8	6 6	3 4	4 4	7 10	6 7	4 1 5 2	1 6 28 2 21 3 12 12
	41	w	6 8	4 6	4 7	4 5	7 8	6 7	4 1 4 2	1 6 28 2 20 6 32 2
	42	w	6 13	5 5	3 3	4 5	3 6	6 7	4 2 4 2	4 6 3 7 22 3 9 2
	43	w	7 14	5 5	3 7	4 5	3 6	7 7	4 1 4 2	1 6 28 2 4 6 3 7
	44	w	7 8	1 5	3 7	5 5	7 8	7 8	4 1 5 2	1 3 22 2 1 6 28 2
	45	w	6 9	1 6	3 3	4 4	3 4	7 7	4 1 5 2	1 6 28 2 4 7 3 2
	46	w	6 8	4 6	3 4	4 5	3 8	6 7	4 1 4 2	NA NA
	47	w	6 13	5 6	3 7	4 5	6 7	6 6	4 1 4 2	4 6 3 7 22 3 9 2
	48	w	7 13	5 5	3 3	4 5	6 7	6 7	4 2 4 2	1 6 28 2 4 6 3 7
		q	**6 7**	**5 6**	**3 7**	**4 5**	**3 7**	**6 7**	**4 1 4 2**	**1 6 28 2 22 3 9 2**
S-5-20 (25.7.2020)	49	w	7 9	5 7	3 3	4 4	7 12	4 6	3 1 4 1	1 6 28 2 16 6 9 2
	50	w	4 9	5 5	3 7	1 4	7 7	4 6	NA NA	NA NA
	51	w	8 9	5 6	3 3	1 4	3 7	6 6	4 1 4 2	4 6 3 14 20 3 8 2
	52	w	4 9	5 5	3 3	1 4	7 7	4 6	3 2 4 1	1 6 28 2 4 7 1 7
	53	w	6 9	5 6	3 7	4 5	3 7	6 6	2 1 4 1	1 6 28 2 12 3 3 3
	54	w	7 7	5 7	3 7	4 4	7 12	4 6	3 2 4 1	1 6 28 2 16 6 9 2
	55	w	4 7	5 14	3 3	1 4	7 7	4 6	3 2 4 1	4 7 1 7 20 3 8 2
	56*	w	6 9	5 6	3 3	4 4	7 7	6 6	4 2 5 2	20 3 8 2 22 3 9 2
	57	w	7 8	2 5	3 3	4 5	7 12	4 6	4 1 5 2	1 6 18 13 1 6 28 2
	58	w	7 7	5 6	3 4	4 5	5 7	6 6	4 1 4 1	1 6 28 2 22 3 9 2
	59	w	7 9	5 7	3 7	4 4	7 12	4 6	3 2 5 1	16 3 9 2 20 3 8 2
	60*	w	6 9	2 6	3 4	4 5	7 9	6 6	4 2 4 2	1 6 28 2 18 3 23 2
		q	**7 9**	**5 6**	**3 7**	**4 4**	**7 7**	**6 6**	**4 1 5 1**	**1 6 28 2 20 3 8 2**

Estimated relatedness of queens: 0.556

[#] Colony "S-5-19b" is also considered in Table S2.7.2

Table S2.7.4

Sample ID	Bee ID	Bee type	A079	AP043	A113	A024	A107	A007	HBTHE02 – HBTHE04	HBC1601 – AC006 – HBC1602 – HBC1605
A-149-19 (20.7.2019)	61	w	4 10	5 6	3 4	4 4	1 7	4 8	3 2 4 1	4 6 3 7 25 3 25 3
	62*	w	8 10	5 7	3 4	4 5	1 9	4 6	3 2 5 1	18 3 11 2 18 3 11 3
	63	w	10 10	2 7	3 3	4 4	5 5	4 6	2 2 3 2	4 6 3 7 18 3 25 2
	64	w	NA NA	NA NA	NA NA	NA NA	1 5	NA NA	NA NA	NA NA
	65*	w	4 8	6 6	3 3	4 4	5 5	4 6	3 2 4 1	14 6 13 2 18 3 13 3
	66	w	4 10	5 6	3 4	4 4	1 7	4 8	4 1 4 1	18 3 25 7 25 3 25 3
	67	w	4 7	5 7	3 3	4 4	5 5	4 8	2 1 4 1	4 6 3 7 13 3 10 2
	68	w	6 10	5 6	3 3	4 5	1 5	1 4	4 1 4 1	1 6 3 3 4 6 3 7
	69	w	4 8	5 6	3 4	4 5	1 9	4 6	4 1 5 1	4 6 3 7 18 3 11 2
	70	w	10 10	2 7	3 3	4 4	5 5	4 6	2 2 3 2	NA NA
	71	w	4 6	5 6	3 3	4 5	1 5	1 4	3 2 4 1	1 6 3 2 4 6 3 7
	72	w	7 10	5 6	3 3	4 4	1 5	4 8	2 1 3 2	4 6 3 7 13 3 10 3
		q	**4 10**	**6 7**	**3 3**	**4 4**	**1 5**	**4 4**	**3 2 4 1**	**4 6 3 7 18 3 25 7**
A-149-20 (19.7.2020)	73	w	8 11	2 6	3 3	NA NA	NA NA	NA NA	4 1 4 2	NA NA
	74	w	8 8	5 7	3 4	4 5	8 11	4 7	4 2 4 2	19 3 21 2 19 3 28 2
	75	w	8 8	5 6	3 3	4 5	6 8	4 8	4 1 4 1	4 6 4 7 19 3 21 7
	76	w	8 8	6 6	3 4	4 5	5 8	4 4	4 1 4 1	19 3 10 2 19 3 21 2
	77	w	8 11	5 6	3 3	4 4	5 8	4 8	4 1 4 2	18 3 10 2 19 3 21 7
	78	w	11 1	2 5	3 4	4 5	6 8	4 7	3 2 4 2	4 6 3 7 19 3 21 7
	79	w	8 11	6 6	3 3	4 5	6 8	7 8	4 1 4 1	4 6 4 16 19 3 21 2
	80	w	8 11	5 6	4 4	1 4	6 9	4 7	2 1 4 1	1 6 4 15 19 3 21 2
	81	w	8 8	6 6	3 4	4 5	8 9	4 6	4 1 4 1	19 3 15 3 19 3 21 2
	82	w	8 11	5 6	3 3	4 5	5 9	4 7	4 1 4 2	18 3 10 2 19 3 21 7
	83	w	8 11	6 7	3 4	4 5	9 11	7 7	4 2 4 2	19 3 21 7 20 3 28 2
	84	w	8 8	5 7	3 4	4 5	8 11	7 7	4 2 4 2	19 3 21 2 19 3 28 2
		q	**8 11**	**5 6**	**3 4**	**4 5**	**8 9**	**4 7**	**4 1 4 2**	**19 3 21 2 19 3 21 7**

Estimated relatedness of queens: 0.116

Table S2.7.5

Sample ID	Bee ID	Bee type	A079	AP043	A113	A024	A107	A007	HBTHE02 – HBTHE04	HBC1601 – AC006 – HBC1602 – HBC1605
A-168-19 (19.7.2019)	85	w	7 8	5 7	3 3	4 4	9 11	6 9	4 1 4 2	22 3 9 2 24 3 28 2
	86	w	6 8	6 7	3 3	1 4	5 9	6 7	4 1 4 1	1 3 28 3 22 3 9 2
	87	w	6 8	1 7	3 4	4 4	5 11	1 7	4 2 4 2	1 7 4 3 16 3 8 3
	88	w	7 8	7 7	3 3	1 4	9 11	6 6	4 1 4 2	1 3 28 3 1 7 4 3
	89	w	6 8	5 6	3 3	4 4	5 9	7 9	4 2 4 2	1 7 4 3 24 3 28 2
	90	w	7 9	2 7	3 3	4 5	4 5	5 6	4 1 5 1	13 3 10 3 22 3 9 2
	91	w	6 8	5 6	3 3	4 5	11 11	6 8	4 1 6 2	13 3 10 3 22 3 9 2
	92	w	6 8	6 7	3 3	4 5	11 15	6 7	4 1 4 2	1 7 4 3 19 3 9 3
	93	w	7 8	6 6	3 3	4 5	5 15	7 7	4 2 4 2	NA NA
	94	w	6 8	5 7	3 3	NA NA	5 9	6 9	NA NA	NA NA
		q	**6 7**	**6 7**	**3 3**	**4 5**	**5 11**	**6 7**	**4 1 4 2**	**1 7 4 3 22 3 9 2**
A-168-20 (13.7.2020)	95	w	6 6	NA NA	3 3	4 5	4 11	4 7	NA NA	1 7 4 3 20 3 23 2
	96	w	6 8	5 7	3 4	4 4	11 12	6 7	1 1 4 1	1 7 4 3 19 3 4 2
	97	w	7 9	2 6	3 3	4 5	4 5	5 6	4 1 5 1	1 7 4 3 13 3 10 3
	98	w	7 9	2 7	3 3	4 5	4 5	5 6	4 1 5 2	13 3 10 3 22 3 9 2
	99	w	6 9	2 6	3 3	4 5	4 5	4 7	4 2 5 2	1 7 4 3 19 6 17 3
	100	w	6 6	5 6	3 3	4 5	4 11	4 7	4 2 4 2	20 3 7 2 22 3 9 2
	101	w	6 9	2 6	3 3	5 5	4 5	5 7	4 1 5 2	1 7 4 3 19 6 17 3
	102	w	6 9	2 7	3 3	5 6	5 6	6 7	4 1 5 1	13 3 10 3 22 3 9 2
	103	w	7 9	7 7	3 3	4 5	5 11	6 6	4 2 5 2	18 3 28 2 22 3 9 2
	104	w	6 8	5 6	3 3	4 5	5 11	6 8	4 2 6 2	13 3 10 3 22 3 9 2
	105	w	6 8	6 6	3 3	4 5	5 15	7 7	4 2 4 2	19 3 9 3 22 3 9 2
	106	w	7 8	6 6	3 3	4 5	11 16	7 8	4 1 4 2	19 3 9 3 22 3 9 2
	107	w	7 8	5 6	3 4	4 4	11 12	7 7	1 1 4 2	19 3 28 2 22 3 9 2
	108	w	6 8	5 6	3 4	4 4	11 11	2 6	4 2 5 2	1 3 22 2 22 3 9 2
		q	**6 7**	**6 7**	**3 3**	**4 5**	**5 11**	**6 7**	**4 1 4 2**	**1 7 4 3 22 3 9 2**

Estimated relatedness of queens: 1

Table S2.7.6

Sample ID	Bee ID	Bee type	A079	AP043	A113	A024	A107	A007	HBTHE02 – HBTHE04	HBC1601 – AC006 – HBC1602 – HBC1605
A-216-19 (20.7.2019)	109	w	7 8	1 7	4 4	4 5	4 8	2 6	3 2 4 3	21 3 9 3 22 3 22 2
	110	w	8 8	1 6	3 3	4 5	8 8	4 7	4 2 5 2	14 4 8 2 22 3 22 2
	111	w	1 8	5 7	3 4	5 5	5 12	6 9	3 2 5 2	4 6 3 7 22 3 22 2
	112	w	1 8	5 7	4 4	5 5	5 11	6 8	4 2 5 2	1 8 3 7 4 6 3 7
	113	w	8 10	5 6	3 4	5 5	10 12	4 6	4 1 4 2	20 3 8 2 22 3 22 2
	114	w	1 10	1 6	3 3	5 5	10 12	1 6	4 2 4 2	NA NA
	115	w	1 10	5 6	3 3	5 5	10 12	1 6	4 2 4 2	NA NA
	116	w	8 11	1 6	4 4	5 5	2 8	4 4	3 2 4 2	4 6 3 7 22 3 22 2
	117	w	1 11	1 6	4 4	5 5	2 12	4 4	3 2 4 2	4 6 3 7 22 3 22 2
	118*	w	7 8	5 7	3 4	4 5	4 8	2 4	3 2 4 3	1 8 3 3 21 3 9 3
	119	w	1 7	5 7	3 4	5 5	6 12	5 6	3 2 4 4	1 8 3 13 22 3 22 2
	120	w	8 10	1 6	3 3	5 5	8 10	6 6	3 2 4 1	20 3 8 2 22 3 22 2
		q	**1 8**	**1 5**	**3 4**	**5 5**	**8 12**	**4 6**	**3 2 4 2**	**1 8 3 7 22 3 22 2**
A-216-20 (13.7.2020)	144	w	1 10	1 6	3 4	5 5	10 12	4 6	3 2 4 1	20 3 8 2 22 3 22 2
	145*	w	8 8	1 5	4 4	4 5	6 12	6 6	3 1 3 2	1 8 3 2 14 3 13 2
	146	w	8 8	1 6	3 3	4 5	8 8	4 7	4 2 5 2	14 4 8 2 22 3 22 2
	147	w	8 11	1 6	4 4	5 5	2 12	4 6	NA NA	NA NA
	148	w	8 11	1 6	4 4	5 5	2 8	4 6	4 2 4 2	1 8 3 7 4 6 3 7
	149	w	8 8	5 7	4 4	5 5	5 12	6 9	4 2 5 2	4 6 3 7 22 3 22 2
	150	d	1	1	3	5	12	4	3 2	NA
	151	d	8	1	4	5	12	4	3 2	22 3 22 2
	152	d	8	1	4	5	8	6	4 2	NA
		q	**1 8**	**1 5**	**3 4**	**5 5**	**8 12**	**4 6**	**3 2 4 2**	**1 8 3 7 22 3 22 2**

Estimated relatedness of queens: 1

Table S2.7.7

Sample ID	Bee ID	Bee type	A079	AP043	A113	A024	A107	A007	HBTHE02 – HBTHE04	HBC1601 – AC006 – HBC1602 – HBC1605
A-203-19 (20.7.2019)	133	w	8 10	5 7	3 3	1 5	8 12	7 7	4 2 4 2	1 3 10 3 21 3 13 2
	134	w	1 8	6 7	3 7	4 5	7 15	8 9	3 2 4 2	13 4 12 2 16 4 9 2
	135	w	1 1	6 6	3 4	1 4	8 11	4 7	2 3 4 2	13 4 12 2 17 3 13 2
	136	w	1 10	2 7	3 3	4 5	3 7	2 8	4 1 4 2	17 3 9 2 21 3 13 2
	137	w	7 8	5 6	3 3	5 5	8 11	7 7	3 2 5 2	20 3 8 2 21 3 13 2
	138	w	1 9	5 7	3 4	4 5	7 11	7 8	3 2 6 1	16 3 10 2 21 3 13 2
	139	w	6 8	6 6	3 4	5 5	7 10	8 8	4 2 5 2	4 3 16 2 21 3 13 2
	140	w	1 7	5 6	3 3	4 5	8 11	7 8	3 2 5 2	13 4 12 2 20 3 8 2
	141	w	8 10	2 7	3 3	4 5	3 7	2 7	3 2 4 1	13 4 12 2 17 3 9 2
	142	w	1 6	2 7	3 3	4 5	7 12	4 7	4 1 4 2	17 3 10 2 21 3 13 2
	143	d	8	7	3	5	8	7	4 2	NA
		q	**1 8**	**6 7**	**3 3**	**4 5**	**7 8**	**7 8**	**3 2 4 2**	**13 4 12 2 21 3 13 2**
A-203-20 (10.7.2020)	121	w	8 9	6 7	3 3	4 5	5 8	4 6	4 2 5 2	21 3 13 2 26 3 25 2
	122	w	1 1	5 6	3 3	4 4	3 7	4 6	4 1 4 2	1 7 3 16 4 6 3 14
	123	w	1 9	7 7	3 4	4 4	3 7	4 6	4 2 4 2	18 3 11 3 21 3 13 2
	124	w	1 1	5 6	3 7	4 5	7 8	4 6	4 1 4 2	4 6 3 14 21 3 13 2
	125	w	8 9	6 7	3 7	4 5	3 5	4 8	4 2 5 2	1 7 3 16 26 3 25 2
	126	w	6 8	6 7	3 3	1 5	3 5	6 8	4 2 5 2	18 3 29 2 21 3 13 2
	127	w	8 9	6 6	3 3	5 5	5 8	4 6	4 2 5 2	1 7 3 16 26 3 25 2
	128	w	1 9	6 6	3 3	4 5	5 8	4 6	4 2 5 2	1 7 3 16 26 3 25 2
	129	w	6 8	6 7	3 7	1 5	3 5	6 8	4 2 5 2	1 7 3 16 18 3 30 2
	130	w	8 9	6 6	3 7	4 5	3 5	4 6	4 2 5 2	1 7 3 16 26 3 25 2
	131	w	8 9	6 6	3 7	4 5	3 5	4 8	4 2 5 2	21 3 13 2 26 3 25 2
	132	w	1 6	6 6	3 7	1 5	3 5	6 8	4 2 5 2	18 3 30 2 21 3 13 2
		q	**1 8**	**6 7**	**3 7**	**4 5**	**3 8**	**6 8**	**4 2 4 2**	**1 7 3 16 21 3 13 2**

Estimated relatedness of queens: 0.546

Table S2.7.8

Sample ID	Bee ID	Bee type	A079	AP043	A113	A024	A107	A007	HBTHE02 – HBTHE04	HBC1601 – AC006 – HBC1602 – HBC1605
C-91-20a (7.4.2020)	153	w	8 8	2 6	3 4	1 6	5 11	4 7	4 1 5 2	4 6 3 7 16 4 24 2
	154*	w	8 8	2 6	3 4	1 5	5 8	4 7	4 1 5 2	4 6 3 7 18 3 25 7
	155	w	1 8	6 6	3 3	1 5	8 11	6 6	2 2 6 2	16 3 10 3 16 4 24 2
	156	w	1 8	2 5	3 3	5 5	11 15	6 9	2 2 5 2	14 6 13 2 16 4 24 2
		q	**1 8**	**2 6**	**3 7**	**1 5**	**8 11**	**6 7**	**2 2 5 2**	**16 4 24 2 18 3 25 18**
C-91-20b (7.7.2020)	157	w	1 8	2 6	3 3	5 5	8 11	6 6	2 2 6 2	18 3 10 3 18 3 25 18
	158	w	1 8	2 5	3 3	5 5	11 15	7 9	2 2 5 2	4 6 17 12 18 3 25 18
	159	w	1 8	2 5	3 7	1 5	11 15	7 9	5 1 5 2	4 6 17 11 16 4 24 2
	160	w	1 8	2 5	3 3	1 5	8 15	7 9	2 2 5 2	4 6 18 11 16 4 24 2
	161	w	1 8	6 6	3 7	5 5	11 11	6 6	2 2 6 2	18 3 10 3 18 3 25 18
	162	w	8 8	6 6	3 7	5 5	8 11	6 7	2 2 6 2	18 3 10 3 18 3 25 18
	163*	w	1 4	2 5	3 7	5 5	5 11	7 7	2 2 3 2	18 3 23 2 18 4 24 2
	164	w	4 8	5 6	3 7	1 5	5 11	7 7	3 1 5 2	18 3 25 18 19 3 23 2
	165	w	1 1	5 6	3 3	5 5	8 15	6 9	2 2 5 2	4 6 17 11 16 4 24 2
	166	w	1 4	2 5	3 7	5 5	5 11	7 7	2 2 3 2	NA NA
	167	w	8 11	2 6	4 7	1 5	8 8	7 13	2 2 6 2	1 6 9 5 18 3 25 18
	168	w	1 1	2 5	3 3	1 5	8 15	6 9	2 2 5 2	4 6 18 11 18 3 25 18
	169	w	1 4	5 6	3 7	5 5	5 8	7 7	3 1 5 2	18 3 25 18 19 3 23 2
	170	w	1 8	5 2	3 3	5 5	11 15	6 9	5 1 5 2	4 6 18 12 18 3 25 18
	171	d	1	2	7	1	11	6	2 2	18 3 25 18
		q	**1 8**	**2 6**	**3 7**	**1 5**	**8 11**	**6 7**	**2 2 5 2**	**16 4 24 2 18 3 25 18**

Estimated relatedness of queens: 1

Table S2.7.9

Sample ID	Bee ID	Bee type	A079	AP043	A113	A024	A107	A007	HBTHE02 – HBTHE04	HBC1601 – AC006 – HBC1602 – HBC1605
C-188-20a (7.4.2020)	172	w	8 8	5 6	3 3	1 4	11 11	6 6	4 1 4 1	18 3 7 2 26 3 25 2
	173	w	8 13	6 6	3 3	4 5	7 8	6 6	2 2 4 2	4 6 14 17 18 3 7 2
	174	w	8 8	5 6	3 3	1 5	4 11	4 6	3 2 4 2	4 6 15 17 18 3 7 2
	175	w	6 8	5 5	3 3	1 4	11 11	6 6	4 1 4 2	4 6 3 6 26 3 25 2
	176	w	8 8	5 6	3 3	1 4	5 7	6 6	3 1 4 1	4 6 3 6 18 3 29 2
	177	w	7 8	1 5	3 3	1 5	7 11	6 6	3 2 4 1	15 3 7 1 18 3 7 2
	178	w	8 9	5 6	3 3	5 5	7 11	6 6	4 1 4 4	16 3 29 2 18 3 7 2
		q	**6 8**	**5 6**	**3 3**	**1 5**	**7 11**	**6 6**	**4 1 4 2**	**4 6 3 6 18 3 7 2**
C-188-20b (6.7.2020)	179	w	7 8	5 5	3 5	1 4	7 13	6 6	4 1 4 1	4 6 4 12 18 3 7 2
	180	w	6 8	5 5	3 3	1 5	3 7	6 8	3 2 4 2	4 6 3 6 14 3 10 3
	181	w	6 7	1 6	3 3	5 5	11 11	6 6	NA NA	NA NA
	182	w	6 7	5 5	3 3	1 5	11 12	5 6	2 1 4 1	14 3 10 3 18 3 7 2
	183	w	6 8	5 6	3 3	5 5	4 7	4 6	3 2 4 2	4 6 14 17 18 3 7 2
	184	w	6 7	1 6	3 3	5 5	11 11	6 6	3 2 4 1	4 6 3 6 15 3 7 1
	185	w	6 8	5 6	3 3	1 5	4 7	4 6	3 2 4 1	4 6 3 6 4 6 15 17
	186	w	6 8	5 6	3 3	5 5	3 7	6 8	3 2 4 1	4 6 3 6 14 3 10 3
	187	w	6 8	5 5	3 3	1 5	3 7	6 8	3 2 4 1	4 6 3 6 14 3 10 3
	188	w	6 8	5 6	3 3	5 5	3 11	6 8	3 2 4 2	4 6 3 6 14 3 10 3
	189*	w	6 7	1 6	3 3	5 5	3 11	4 6	4 1 4 2	1 6 4 14 4 6 4 6
	190	w	6 7	6 6	3 3	5 5	6 11	1 6	4 2 4 2	1 3 11 3 4 6 3 6
	191	w	8 8	2 5	3 6	1 5	10 11	1 6	4 1 4 4	18 3 7 2 18 3 30 2
	192	w	6 8	5 6	3 3	1 5	3 7	6 8	3 2 4 2	4 6 3 6 14 3 10 3
		q	**6 8**	**5 6**	**3 3**	**1 5**	**7 11**	**6 6**	**4 1 4 2**	**4 6 3 6 18 3 7 2**

Estimated relatedness of queens: 1

Chapter three

Reduced parasite burden in feral honeybee colonies

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Abstract

Bee parasites are the main threat to apiculture, and since many parasite taxa can spill over from honeybees (*Apis mellifera*) to other bee species, honeybee disease management is important for pollinator conservation in general. It is unknown whether honeybees that escaped from apiaries (i.e., feral colonies) benefit from natural parasite-reducing mechanisms like swarming or suffer from high parasite pressure due to the lack of medical treatment. In the latter case, they could function as parasite reservoirs and pose a risk to the health of managed honeybees (spillback) and wild bees (spillover).We compared the occurrence of 18 microparasites among managed (N = 74) and feral (N = 64) honeybee colony samples from four regions in Germany using qPCR. We distinguished five colony types representing differences in colony age and management histories, two variables potentially modulating parasite prevalence. Besides strong regional variation in parasite communities, parasite burden was consistently lower in feral than in managed colonies. The overall number of detected parasite taxa per colony was lower, and Trypanosomatidae, chronic bee paralysis virus, and deformed wing viruses A and B were less prevalent and abundant in feral colonies than in managed 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. Synthesis and application: Our study confirms the hypothesis that the natural mode of colony reproduction and dispersal by swarming temporally reduces parasite pressure in honeybees. We conclude that feral colonies are unlikely to contribute significantly to the spread of bee diseases. There is no conflict between the conservation of wild-living honeybees and the management of diseases in apiculture.

Introduction

Disease transfer between managed and wild animals is a potential source of conflict between the livestock sector and nature conservation (Mysterud & Rolandsen, 2019). Populations managed in intensive animal husbandry can be vulnerable to diseases transmitted from wild populations because high animal densities and low genetic variance increase the risk of epidemics (Gortázar et al., 2007). Conversely, diseases of livestock can become a threat to wildlife, e.g., when managed species introduce diseases into non-native areas (Schommer & Woolever, 2008; Ayala, Yabsley & Hernandez, 2020; Costanzi et al., 2021). An interesting example of potentially negative livestock-wildlife interactions is that of the disease associations between managed western honeybees (*Apis mellifera*), wild-living honeybees, and other bee species (Fürst et al., 2014; Ravoet et al., 2014). The Western honeybee is native to Africa, Europe, and Western Asia, and is an important pollinator of wild plants both in its native and introduced range (Dick, 2001; Hung et al., 2018). The species is managed by beekeepers globally to pollinate the flowers of crops and to produce honey and other hive products (Crane, 1990; Rollin & Garibaldi, 2019). Where self-sustaining wild populations have gone extinct, as in many regions of Europe and Western Asia (Pirk, Crewe & Moritz, 2017; Requier et al., 2019a), apiculture also serves to protect the honeybee as a species. However, modern rationalized beekeeping can conflict with conservation (Geldmann & González-Varo, 2018; Iwasaki & Hogendoorn, 2022; Panziera et al., 2022). By preventing swarming and maintaining unnaturally large, continuously breeding colonies; by crowding hives in apiaries, and by seasonally moving colonies between bee yards, apiculture promotes the reproduction and spread of bee parasites (Seeley & Smith, 2015; Loftus, Smith & Seeley, 2016; Brosi et al., 2017; Nolan & Delaplane, 2017; Peck & Seeley, 2019; Martínez-López, Ruiz & De la Rúa, 2022). On a global scale, the transport of hives and their products can expose the bees to entirely novel parasites (Goulson & Hughes, 2015). This was famously demonstrated by the worldwide invasions of western honeybee populations by the ectoparasitic mite *Varroa destructor,* whose natural host was the eastern honeybee *A. cerana* (Kraus & Page, 1995; Wilfert et al., 2016; Traynor et al., 2020). Unfortunately, many bee parasites are not restricted to a single species. Since honeybees inevitably share floral resources with other pollinators, their parasites can spill over to, and harm, populations of other bee species (Fürst et al., 2014; Burnham et al., 2021; Piot et al., 2022; Tehel et al., 2022). Therefore, managing honeybee diseases is important for the conservation of bee pollination services in general, both in the contexts of crop production and ecosystem functioning (Brosi et al., 2017; Bartlett, 2022).

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 (Schmid-Hempel, 1998). However, parasites are not considered to be a limiting factor for wild honeybee

populations under natural conditions (Bailey, 1958; Ratnieks & Nowakowski, 1989; Fries & Camazine, 2001; Fries, Lindström & Korpela, 2006). An important reason seems to be the regulation of parasite populations within colonies as a side-effect of the bees' natural cycle of colony reproduction and dispersal (Loftus, Smith & Seeley, 2016; DeGrandi-Hoffman, Ahumada & Graham, 2017). Honeybee 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 (Seeley, 2010). The swarming bees do not transfer brood to the new nesting site, 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 (Royce et al., 1991; Loftus, Smith & Seeley, 2016). Furthermore, the rate at which new parasite species enter wild colonies is most probably lower compared to the situation at apiaries, since their nests are typically widely dispersed in the landscape (Lindström, Korpela & Fries, 2008; Seeley & Smith, 2015; Nolan & Delaplane, 2017). Besides these ecological factors, wild honeybee populations are more resilient against disease than managed populations because they are more likely to evolve defences against parasites via natural selection (Neumann & Blacquière, 2017; Pirk, Crewe & Moritz, 2017). A famous example is a population of non-native wild honeybees, inhabiting forests in the species' introduced range in the north-eastern USA, which was subjected to rapid evolution upon the arrival of *Varroa destructor* in the 1980s and remains viable (Mikheyev et al., 2016; Seeley, 2017). Not only do wild honeybee populations deliver free pollination services (Chang & Hoopingarner, 1991), but they can also benefit the beekeeping sector when genetic adaptations to (novel) stressors, including parasites, are passed on to the managed population (Pirk, Crewe & Moritz, 2017). Self-sustaining wild honeybee populations exist both in the species' native range in Africa (Dietemann, Pirk & Crewe, 2009) and in its introduced range in Australia (Oldroyd et al., 1997), and parts of the Americas (Winston, 1992; Seeley, 2007).

The situation might differ for wild-living honeybee colonies which are recent escapees from apiaries. For example, in Germany, despite active swarm prevention by beekeepers, thousands of honeybee swarms emigrate from bee yards each spring and colonise tree cavities in managed forests. However, their annual survival rate is far below the threshold required to maintain a self-sustaining population (Kohl, Rutschmann & Steffan-Dewenter, 2022). We here refer to such honeybees as "feral" (Daniels & Bekoff, 1989), as opposed to "wild", regardless of whether the native or introduced range of the species is considered. Feral colonies exist wherever apiculture is practised, and they are probably also widespread in Europe (Browne et al., 2020; Dubaić et al., 2021; Kohl et al., 2022; Kohl & Rutschmann, 2018; Oleksa et al., 2013; Rutschmann et al., 2022; Thompson, 2012). On the one hand, feral colonies might have a lower parasite burden than managed colonies due to the natural parasite-reducing effects of swarming and the dispersal of

nest sites. On the other hand, with an evolutionary background of artificial selection and a history of care by beekeepers, feral colonies are likely to be stressed by multiple environmental factors and might develop high parasite loads without medical treatment (Thompson et al., 2014).

Understanding the disease ecology of recently feralised honeybee colonies is important because they create a potential management conflict. Honeybees are usually considered livestock animals and beekeepers are obligated to register their hives, regularly apply miticide treatments, and report infectious diseases to prevent epidemics. If feral colonies carry high loads of parasites, however, they might reinfect managed colonies (spillback), undermining the veterinary measures undertaken to combat disease (Frey & Rosenkranz, 2014; Thompson et al., 2014). When highly infected feral colonies disperse into natural areas, they might also function as vectors for parasites that can spill over to non-*Apis* wild bees (Fürst et al., 2014; Burnham et al., 2021; Piot et al., 2022; Tehel et al., 2022). This situation would suggest management options aiming at preventing feralisation or eradicating feral colonies (Taylor et al., 2007). Conversely, where the Western honeybee is a native species, promoting or re-establishing populations of wild-living honeybees is a legitimate conservation goal (Requier et al., 2019a; Panziera et al., 2022). It would then be inappropriate to combat feral colonies since they can be the source of future wild populations.

To assess whether feral honeybees might 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 (D'Alvise et al., 2019). 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 (Youngsteadt et al., 2015). 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 (D'Alvise et al., 2019; Faurot-Daniels et al., 2020). 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 (Garbuzov et al., 2020) increases the risks of parasites transmissions, and parasite loads in summer affect subsequent colony winter survival (Ravoet et al., 2013). In addition to the difference between managed and feral, we distinguished five colony types representing different colony age classes and management histories since these factors potentially modulate parasite prevalence. The collected data allowed us to make a nuanced assessment of the effect of environmental differences between managed and feral honeybee colonies on parasite prevalence.

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 3.1) in July 2020. In the first three study regions, feral colonies were found during a population-monitoring study that was based on systematic censuses of tree cavities made by the black woodpecker, which are the primary nesting sites of honeybee colonies in managed forests in Germany (Kohl & Rutschmann, 2018; Kohl, Rutschmann & Steffan-Dewenter, 2022). We sampled from all detected feral colonies whose recent history was known to us (see next paragraph). In the fourth study region (Munich), we were able to select 15 out of approx. 80 feral honeybee nest sites that had previously been mapped by two of us using a combination of private search and citizen reports collected via the BEEtree-Monitor network (S. Roth and F. Remter, unpublished). We selected the 15 nest sites for sampling based on the criteria of accessibility and knowledge of the recent occupation history, and to assure an even distribution over the city and an equal representation of nests in trees and buildings. To obtain locations of managed colonies, we contacted beekeepers via the local beekeeping organizations and asked for permission to sample bees from their hives. This resulted in a sample of managed colonies that roughly matched the sample of feral colonies in terms of size and spatial distribution (Figure. 3.1). The sampled feral colonies nested in tree cavities or building walls with entrance heights between 2–18 m above the ground. Except for four of the 64 colonies, which had neighbouring colonies in the same tree or building wall, feral colonies were spatially separated from others. The sampled managed colonies were kept in hives and were usually placed next to other colonies in apiaries. The median number of colonies at the apiaries was six (range: 1–28 colonies), which well represents the typical apiary size of German beekeepers, who mostly practice beekeeping as a hobby or sideline occupation (Deutscher Imkerbund, 2020).

A factor potentially affecting parasite burden is colony age. Therefore, we noted for each colony whether it had overwintered at least once (age >1 year) or had been newly founded in the year of sampling (age <1 year). Amongst feral colonies 72% (N = 46) were recent founders (swarms that moved into the cavity and founded a colony in spring), and 28% (N = 18) had overwintered at least once. Amongst managed colonies we sampled similar numbers of overwintered (N = 38; 51%) and newly founded colonies (N = 36; 49%), since we assumed the ratio of old versus young colonies at apiaries in summer is about 50:50. In the group of young managed colonies, we further distinguished between nucleus colonies, i.e., daughter colonies created by taking brood frames 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 transferring them into a new hive together with a queen (N = 2).

At least 20 bees per colony were captured by placing a white butterfly net in front of the hive or cavity entrance (see supplementary information in chapter two, for details of the sampling method). 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.

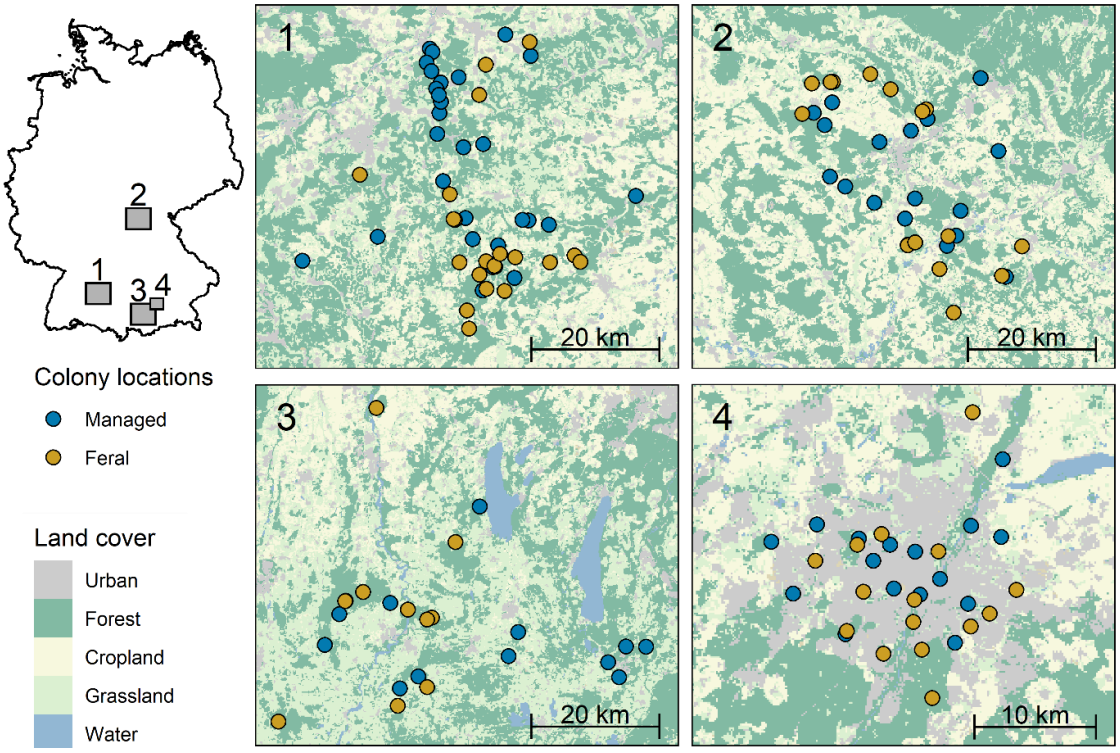

Figure 3.1: Geographic locations of the sampled managed (N = 74) and feral (N = 64) honeybee colonies in four regions in southern Germany (1: Swabian Alb, 2: Counties Coburg and Lichtenfels, 3: County Weilheim-Schongau, 4: City of Munich). Note that the map of Munich has a different scale. Land cover data are from Weigand et al. (2020).

Parasite testing

We assessed the colony-level occurrence of 18 microparasites using high-throughput qPCR on a Biomark HD system (Standard BioTools, San Francisco, CA) with parasite-specific primers (Evans, 2006; Lourenço et al., 2008; Budge et al., 2010; Martínez et al., 2010; Locke et al., 2012; Papp, Spann & Marschang, 2014; Cepero et al., 2015; D'Alvise et al., 2019) closely following an established protocol (D'Alvise et al., 2019), 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 S3.1 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, $\log_{10}(n/100\text{ ng RNA}+1)$, as the "parasite load".

Statistical analyses

All statistical analyses were performed with R version 4.0.5 (R Core Team, 2022) and figures were created using "ggplot2" (Wickham, 2016). We analysed whether the number of detected parasite taxa per colony (univariate analysis) and the microparasite community composition (multivariate analysis) differed depending on "management" (two levels: "managed" versus "feral") and "colony type" (five levels: "managed: overwintered", "managed: nucleus colonies", "managed: hived swarms", "feral: overwintered" and "feral: founders"). In all four analyses, we accounted for the potential effect of spatial location on microparasite communities.

We analysed the number of parasite taxa detected (count data) using generalized linear models (function "glmmTMB"; Brooks et al., 2017, 2019). Besides the main factors of interest, "management" or "colony" type", we included "region" (four levels) as well as the interaction between "region" and "management" or "colony type" as additional predictors to account for spatial differences and for potential variation in the effect of management or colony type between regions. Considering "region" to describe the spatial component was reasonable since most spatial variation in parasite assemblages was attributable to variation between regions rather than variation between sites within regions (see below). Count data can be analysed with a range of model types assuming different probability distributions (Brooks et al., 2017). We therefore first created five models with the same predictor formula but different family functions in "glmmTMB" (family = "poisson", "nbinom1", "nbinom2", "compois" or "genpois") and selected the best models using the Akaike information criterion for small sample sizes (AICc, function "AICctab" from the "bbmle" package; Bolker, 2022). In both comparisons (factor "management" or "colony type") models with a generalized Poisson distribution (family = "genpois") had the lowest AICc ($\Delta AICc \geq 12$) so we used these for the analyses. We then tested for significant deviations from model assumptions using the functions "simulateResiduals", "testResiduals" and "testCategorical" from the "DHARMa" package (Hartig, 2022). No significant deviations were found in tests for uniformity, dispersion, and outliers, but Levene's test detected significant differences in variance of parasite counts between the four study regions. This problem could be fixed by adding a formula for dispersion with "region" as the single fixed effect. The final

formulations of the full models were "glmmTMB(*Number of parasites* ~ X + region + X : region, dispformula = ~ region, family = genpois)" with "X" denoting either "management" or "colony type". Predictions of the mean number of parasite taxa and 95% confidence limits were produced using the function "emmeans" (Lenth, 2022). To test whether management and colony type or their interactions with region significantly affected parasite counts, we compared pairs of nested models (where one model contained, and the other missed, the predictor of interest) using likelihood ratio tests (LRT, function "anova"). We assessed the effect of management or colony type while accounting for the effect of region (see supplementary information, Tables S3.2–4, S3.11 and S3.12 for the specifications of the nested models). In the case of the five-level factor "colony type", we used the function "glht" from the "multcomp" package (Hothorn, Bretz & Westfall, 2008) for Tukey post hoc tests of pair-wise differences in parasite numbers between colony types.

To analyse differences in parasite community composition, we used distance-based redundancy analyses with "management" or "colony type" as the constraining factor (dbRDA, function "db.rda" from the "vegan" package, Oksanen et al., 2022). 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 ("constraints"). 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; Borcard & Legendre, 2002; Oksanen et al., 2022). The model formulations were "db.rda(*Jaccard distance matrix of parasite communities* ~ X + Condition(scores(pcnm(*distance matrix of sample locations*)))", with "X" denoting either "management" or "colony type". 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 (formulation: "db.rda(*Jaccard distance matrix of parasite communities* ~ region)") showed that 14.9% of the variation could be attributed to differences between the four regions (see Figure S3.1). 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 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 (Blaker, 2000; Stevenson et al., 2022) as well as the mean colony-level loads.

Results

The number of microparasite taxa detected per colony ranged between one and nine, with an overall average of 5.8 taxa. Parasite counts differed significantly between regions (LRT: D.f. = 6, $\chi^2 = 26.819$, $P = 0.00016$, Table S3.2); they were lowest in Coburg and Lichtenfels (mean: 4.9), intermediate on the Swabian Alb (mean: 5.7), 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, $\chi^2 = 14.677$, $P = 0.00013$, Table S3.3). Feral colonies had, on average, one parasite less (median: 5, mean: 5.4, range 1–8) than managed colonies (median: 6, mean: 6.2, range 4–9) (Figure 3.2a). The difference between feral and managed colonies was consistent across study regions (no significant interaction between management and region; LRT: D.f. = 3, $\chi^2 = 0.947$, $P = 0.814$, Table S3.4).

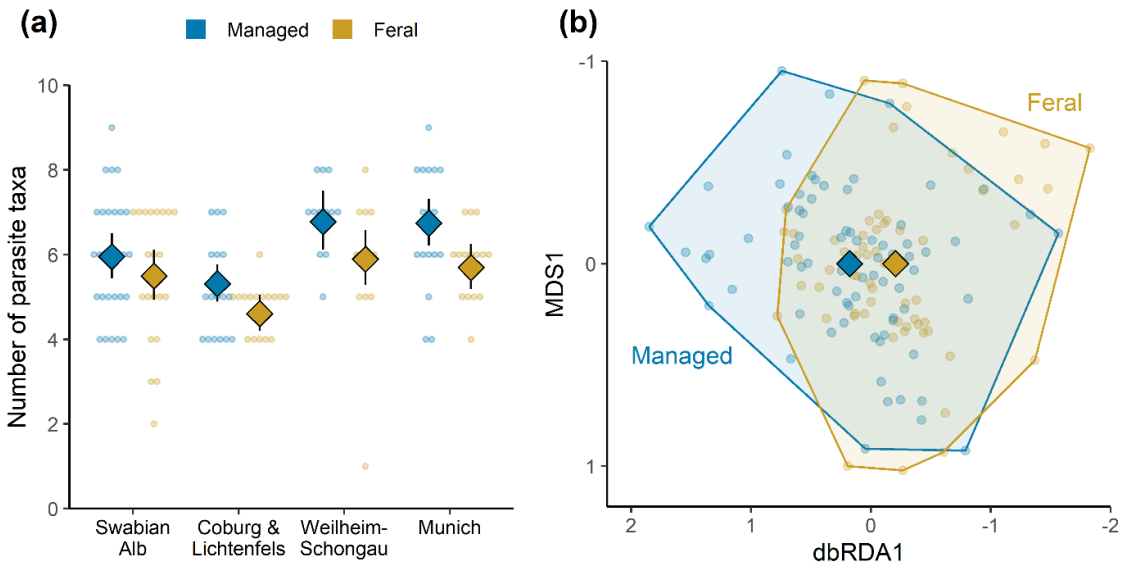

Figure 3.2: Comparison of parasite burden between managed (N = 74) and feral (N = 64) honeybee colonies. **(a)** Number of microparasite taxa detected among the 18 taxa assayed for each of the four study regions. Dots are raw data; diamond symbols and vertical lines give model-estimated means and 95% confidence intervals. See Table S3.5 for an overview of model predictions. **(b)** Representation of 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.

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. 3.2b). 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 3.3). 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 abundances tending to be lower in feral colonies (Figure 3.4a). Considering only colonies which were tested positive showed that parasite loads of infected feral and managed colonies were generally similar (Figure 3.4b).

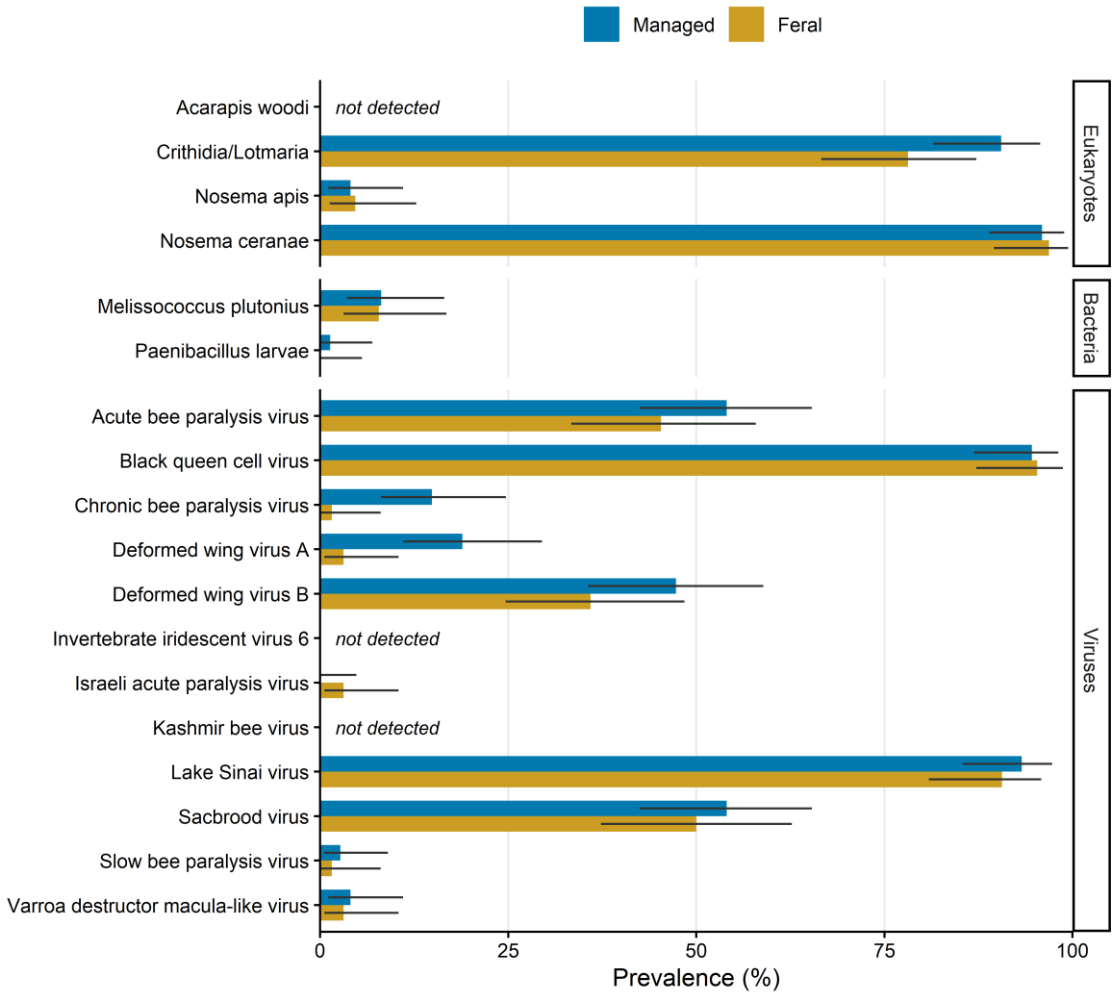

Figure 3.3: Prevalence (percentage of colonies tested positive; bars) and 95% confidence intervals (black lines) for 18 microparasite taxa among managed (N = 74) and feral (N = 64) honeybee colonies. See Table S3.6 for an overview of prevalence values and Tables S3.7–10 for overviews of parasite prevalence divided by study region.

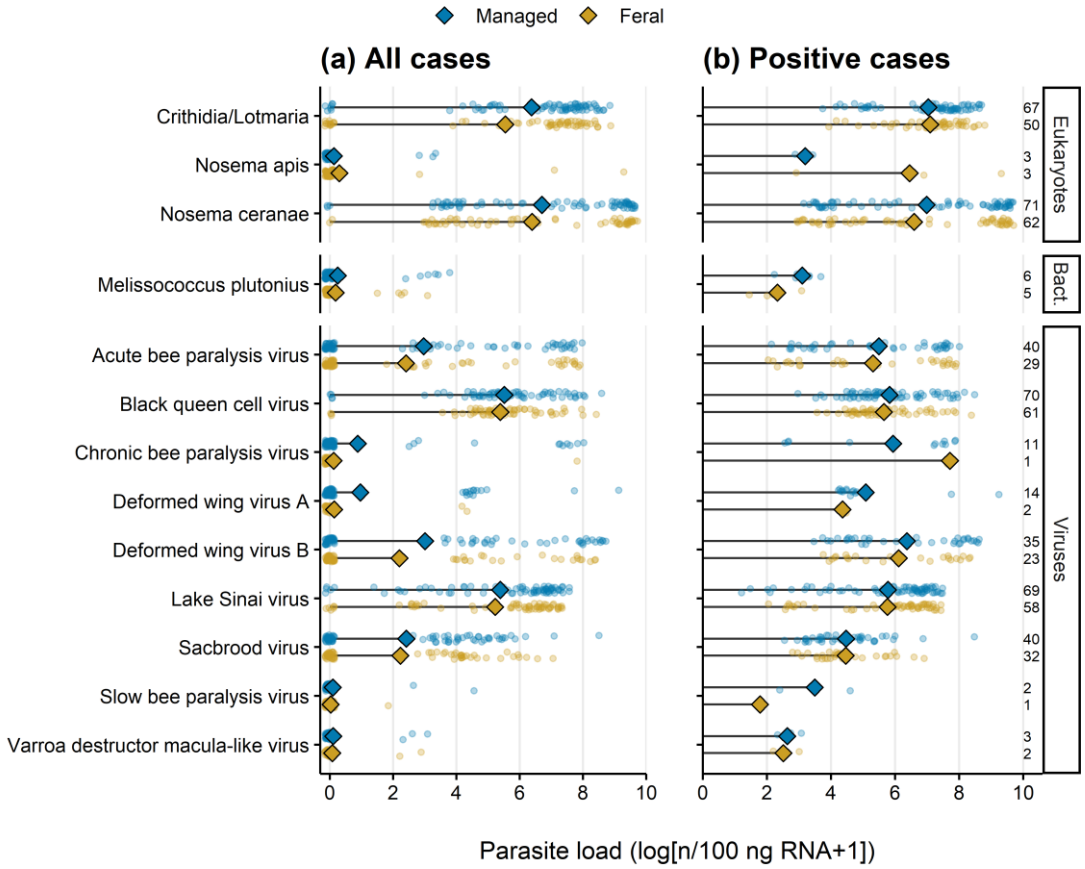

Figure 3.4: Parasite loads of managed and feral honeybee colonies. Only microparasite taxa detected at least once in each group are shown. **(a)** Parasite loads of individual colonies (dots; random jitter is added to reduce overplotting) and mean parasite loads (diamonds) based on all colonies tested (managed: N = 74, feral: N = 64). **(b)** Parasite loads based on positive cases only. Dots are parasite loads of individual colonies, diamonds are means, and numbers denote sample sizes. See Table S3.6 for an overview of parasite load values and Tables S3.7–10 for overviews divided by study region.

An analysis of the number of parasites in relation to five colony types gave a more nuanced picture of differences in parasite burden (LRT, factor "colony type" after "region": D.f.: 4, $\chi^2 = 23.23$, $P = 0.0001$, Table S3.11). 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 3.5a). These differences were largely consistent across study regions (no significant interaction between colony type and region; LRT: D.f.: 12, $\chi^2 = 9.546$, $P = 0.656$, Table S3.12; see Figure S3.2 and Table S3.13 for parasite counts divided by colony type and study region). A pairwise comparison revealed that the difference between feral founders and overwintered managed colonies ($P<0.001$) and between feral founders and hived swarms ($P = 0.002$) were statistically significant.

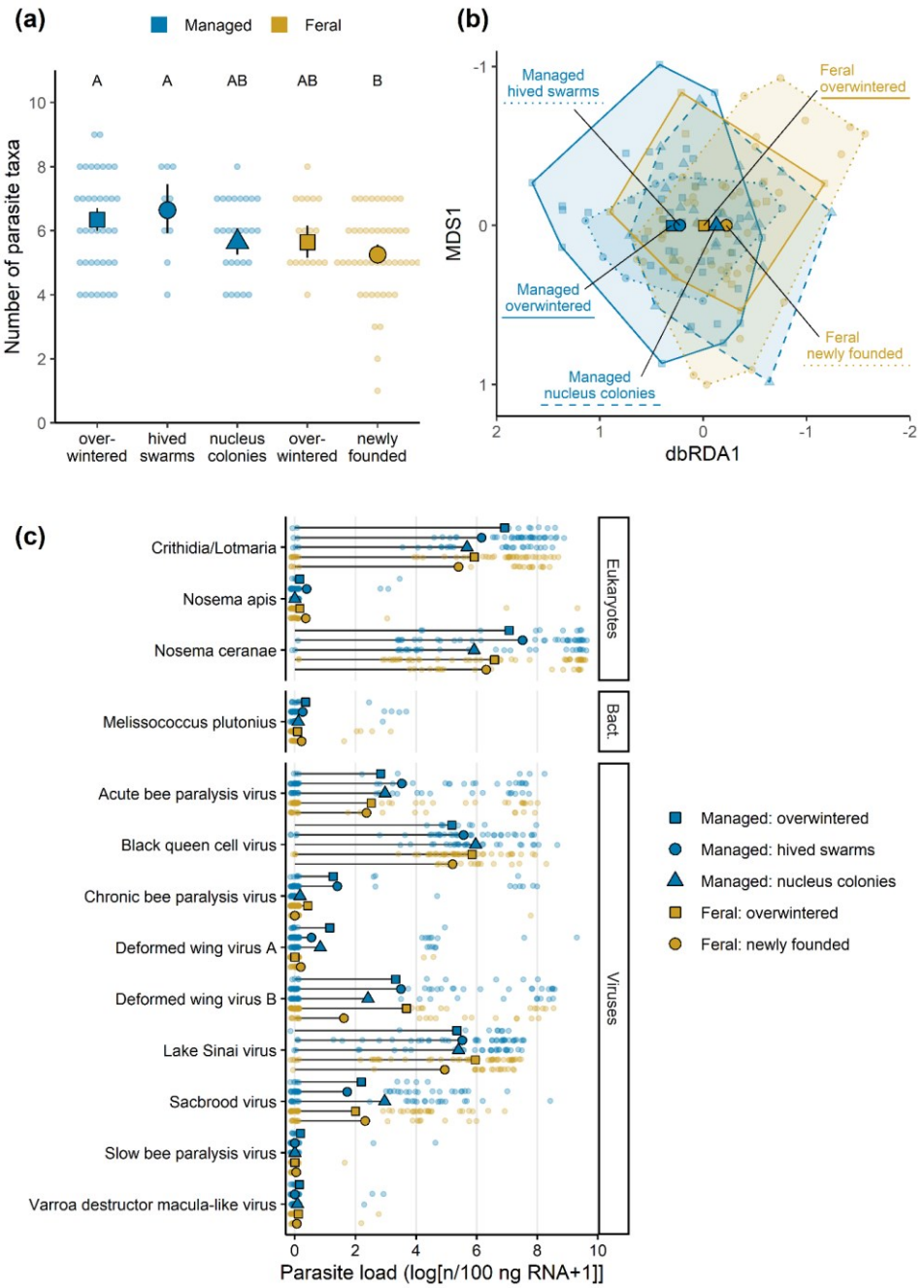

Figure 3.5: Comparison of parasite burden between different types of managed and feral honeybee colonies **(a)** Number of microparasite taxa detected among the 18 taxa assayed. Dots are raw data; large symbols and vertical lines give model-estimated means and 95% confidence intervals. Pairs that do not share a letter differ significantly (P<0.05). **(b)** Relative differences in microparasite community composition between the five colony types as revealed by a distance-based redundancy analysis with colony type as the constraining factor (spatial structure partialled out). The primary dbRDA-axis separating the colony types (1.9% of total variation) is shown along the first unconstrained axis (MDS1, 16.4% of the variation). Dots represent locations of individual colonies and diamonds are mean locations. **(c)** Parasite loads in relation to colony type. Dots are parasite loads of individual colonies and diamonds are means. Only microparasite taxa detected at least once among managed colonies and among feral colonies are shown (same selection as in Figure 3.4). See Table S3.14 for an overview of parasite prevalence and parasite loads by colony type.

There were also differences in the colony-level composition of parasite taxa between the five colony types (colony type explained 3.3% of parasite community variation according to dbRDA; permutational anova: $P = 0.099$; Figure 3.5b). The differences in parasite communities, albeit marginal, resembled the differences in parasite counts: along the first dbRDA-axis, parasite assemblages of feral founders were separated from those of hived swarms and overwintered managed colonies, while the parasite communities of overwintered feral colonies took an intermediate position, and the parasite communities of managed nucleus colonies were closer to those of feral colonies than to those of other managed colonies (Figure 3.5b). Accordingly, mean colony-level loads of individual parasite taxa 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 3.5c).

Discussion

We compared the parasite burden of honeybee colonies in managed and feral conditions to evaluate whether colonies that escaped from apiaries pose a risk to managed and wild 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.

We considered parasite burden based on the prevalence of 18 microparasites determined using qPCR. This means that we did not cover all known bee parasites. We also cannot exclude the possibility of some false non-detections of targeted RNA viruses due to their high evolutionary rates. However, our conclusions about relative differences in parasite burden between groups are robust because all colonies were subjected to the same qPCR-assays and because groups were evenly distributed across study regions.

Comparing colonies from four regions in southern Germany revealed strong geographic differences in parasite numbers and parasite community composition. These differences might be explained by a combination of factors including managed colony density, land use, climate, and colonization histories of individual parasites. The management implication is that moving managed hives, even over moderate distances (the distance between our study regions Weilheim-Schongau and Munich is approx. 50 km), bears the risk of introducing bee parasites that would otherwise not be present locally. Considering the potential negative effects on local wild bee communities and on honeybees managed by non-migratory beekeepers (Martínez-López, Ruiz &

De la Rúa, 2022), apicultural disease management needs to pay more attention to regional differences in parasite communities.

Although region of sampling had a stronger effect on parasite communities than management, we believe that the differences found between feral and managed colonies are ecologically relevant. With about six parasite taxa detected per colony on the overall average, the reduced count of 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 (vanEngelsdorp et al., 2009), although this relationship might be non-linear (Ravoet et al., 2013). Analysing the associations between 18 parasite taxa and colony mortality, Ravoet et al. (2013) found that winter mortality steeply increased with the number of parasite species rising from three to five, but additional parasites had no further effect. In our study, the percentage of colonies with more than five 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, it was not entirely random which parasites were less prevalent in feral colonies: 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 (Tehel, Brown & Paxton, 2016; Strobl et al., 2019). The two trypanosomatid species *Chrithidia mellificae* and *Lotmaria passim* (among which we did not distinguish with our PCR assay) are unicellular parasites that colonise the bees' hindgut (Schwarz et al., 2015). They have been experimentally shown to reduce the lifespan of individual workers (Strobl et al., 2019) and their presence is associated with colony-level winter mortality (Ravoet et al., 2013). 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 (Ribière, Olivier & Blanchard, 2010). Interestingly, CBPV seems to be an emerging threat, as indicated by a rapid rise of cases among Britain's apiaries during the last decade (Budge et al., 2020). 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) genotypes A and B in feral compared to managed colonies is, ecologically, the most important detected difference since DWV are among the main causes of winter colony losses (Genersch et al., 2010; de Miranda & Genersch, 2010; Dainat et al., 2012a). 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 (de Miranda & Genersch, 2010). 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 (Dainat et al., 2012a). 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* (de Miranda & Genersch, 2010; Paxton et al., 2022). 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 (McMahon et al., 2016; Paxton et al., 2022). In turn, the mite's reproduction is enhanced by the presence of DWV, making the mite-virus pair a deadly symbiosis (Di et al., 2016). 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 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 (Dietemann et al., 2013), but this was not possible in the case of the feral colonies. However, since DWV and *Varroa* correlate (Dainat et al., 2012b; Norton et al., 2021), the lower prevalence of DWV 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. 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 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 3.5c). 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 as a whole because only about 10% of the feral colonies present in summer are older than one year (Kohl, Rutschmann & Steffan-Dewenter, 2022), 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 demography 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 (Kohl, Rutschmann & Steffan-Dewenter, 2022), and therefore, their reduced

parasite burden needs to be explained by ecological/environmental, rather than genetic, differences from managed honeybees. By considering five colony types representing differences in colony age and management history (Table 3.1), 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 (Royce et al., 1991; Loftus, Smith & Seeley, 2016; DeGrandi-Hoffman, Ahumada & Graham, 2017). 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, 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 resemble those of established (old) colonies. Indeed, parasite counts did not 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. The latter might be explained by the fact that beekeepers usually manage established colonies in such a way that brood is continuously produced, while managed nucleus colonies and overwintered feral colonies typically experience a brood pause in spring and thus a temporal reduction of the breeding ground for parasites (Table 3.1) (Loftus, Smith & Seeley, 2016). Importantly, young managed colonies founded by swarms ("hived swarms") were infested by a significantly higher number of microparasite taxa than young feral colonies. This contrast needs to be regarded with care since our sample size of hived swarms was low (N = 9), but it suggests that not only differences in population age structure between managed and feral colonies, but also in the environment, contribute to the difference in 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 spatially dispersed (Lindström, Korpela & Fries, 2008; Seeley & Smith, 2015; Nolan & Delaplane, 2017). The crowding of colonies promotes random drifting and robbing behaviour, and both drifters and robbers can transfer parasites between hives (Lindström, Korpela & Fries, 2008; Seeley & Smith, 2015; Peck & Seeley, 2019). Another highly consistent difference is that beekeepers conventionally keep their hives at ground level, while feral colonies typically nest in cavities several metres above the ground. The bees' preference for aerial cavities is thought to serve as a

Table 3.1: 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.

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 the 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 whether it is possible to restore the natural parasite-reducing effect of swarm-founding under apicultural management.

Feral honeybee colonies carrying many parasites and/or high parasite abundances would be a nuisance to apicultural disease management and would pose a risk to the health of non-*Apis* wild bees. 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 mechanism of parasite reduction that might also be effective in other swarm-founding social insects (McGlynn, 2012). Given that feral colonies have a relatively low parasite burden and that they make up a small fraction of the overall honeybee population (in Germany feral colonies make up about 5% of the whole honeybee population in summer), we conclude that it is unlikely that they 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 (Taylor et al., 2007). In fact, our data suggest that there is no conflict between the promotion of wild-living 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 and to reduce the risk of disease spread by apiculture (Loftus, Smith & Seeley, 2016; Dynes et al., 2019; Büchler et al., 2020).

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Supplementary information

RNA extraction and qPCR analyses

We obtained colony-level total RNA by extracting it from multi-bee homogenates using a TRIzol protocol. For each colony, 20 workers were placed in a 15 mL reaction tube with one 0.25-inch ceramic bead (MP Biomedicals), five 2.8-mm Precellys® steal beads, 0.7 g 0.1-mm glass/zirconia

beads (BioSpec) and 4 mL TRIzol (Invitrogen). The mixtures were homogenized with a FastPrep24 (MP Biomedicals) running two times at 6 m/s speed for 60 s (in between the runs, tubes were inverted and vigorously shaken by hand to remove bees stuck to the bottom of the tubes). The homogenates were incubated for five minutes at room temperature (RT), mixed with 800 µL chloroform, vigorously shaken for 15 s, and incubated for another five minutes at RT. After 15 min of centrifugation at 12,000 g and 4°C, 200 µL of the aqueous phases were transferred to 1.5-mL reaction tubes and mixed with 250 µL isopropanol by repeated inverting. After another incubation for 10 min at RT, the precipitated RNA was separated by centrifugation (12,000 g, 4°C). The resulting supernatants were removed, and the RNA pellets were washed with 75% ethanol, dried for 5 min at RT, and redissolved in 50 µL nuclease-free water. RNA concentrations (determined using a NanoDrop spectrophotometer, Themo Fisher) ranged between 2215 and 6117 ng/µL (mean: 3519 ng/µL). They were used as references for calculating colony-level parasite loads. Production of cDNA, pre-amplification of target sequences, qPCR on a Biomark HD system, and calculation of number of target molecules from Cq-values were performed as described in D'Alvise et al. (2019).

Table S3.1: Overview of the primers used in this study.

PCR target	Primers 5'–3'	Reference
Acarapis woodi	F: GGAATATGATCTGGTTTAGTTGGTC R: GAATCAATTTCCAAACCCACCAATC	Cepero et al., 2015
Crithidia/Lotmaria	F: CCGCTTTTGGTCGGTGGAGTGAT R: GCAGGGACGTAATCGGCACAGTTT	D'Alvise et al., 2019
Nosema apis	F: CAGTTATGGGAAGTAACATAGTTG R: CGATTTGCCCTCCAATTAATCTG	D'Alvise et al., 2019
Nosema ceranae	F: TGAGGCAGTTATGGGAAGTAATATTATATTG R: ACTTGATTTGCCCTCCAATTAATCAC	D'Alvise et al., 2019
Bacteria		
Melissococcus plutonius	F: TGTTGTTAGAGAAGAATAGGGGAA R: CGTGGCTTTCTGGTTAGA	Budge et al., 2010
Paenibacillus larvae	F: CGGGAGACGCCAGGTTAG R: TTCTTCCTTGGCAACAGAGC	Martínez et al., 2010
Viruses		
Acute bee paralysis virus	F: TCATACCTGCCGATCAAG R: CTGAATAATACTGTGCGTATC	Locke et al., 2012
Black queen cell virus	F: AGTGGCGGAGATGTATGC R: GGAGGTGAAGTGGCTATATC	Locke et al., 2012
Chronic bee paralysis virus	F: CAACCTGCCTCAACACAG R: AATCTGGCAAGGTTGACTGG	Locke et al., 2012
Deformed wing virus A	F: TTCATTAAAGCCACCTGGAACATC R: TTTCCTCATTAACTGTGTCGTTGA	Locke et al., 2012
Deformed wing virus B	F: GCCCTGTTCAAGAACATG R: CTTTTCTAATTCAACTTCACC	Locke et al., 2012
Invertebrate iridescent virus 6	F: TGGTTYACCCAAGTACCKGTTAG R: ATGCKGACCATTCGCTTC	Papp et al., 2014
Israeli acute paralysis virus	F: CCATGCCTGGCGATTCAC R: CTGAATAATACTGTGCGTATC	Locke et al., 2012
Kashmir bee virus	F: CCATACCTGCTGATAACC R: CTGAATAATACTGTGCGTATC	Locke et al., 2012
Lake Sinai virus	F: TCATCCCAAGAGAACCAC R: GCATGGAAGAGAGTAGGTA	D'Alvise et al., 2019
Sacbrood virus	F: TTGGAACTACGCATTCTCTG R: GCTCTAACCTCGCATCAAC	Locke et al., 2012
Slow bee paralysis virus	F: GCGCTTTAGTTCAATTGCC R: ATTATAGGACGTGAAAATATAC	Locke et al., 2012
Varroa destructor macula-like virus	F: ATCCCTTTTCAGTTCGCT R: AGAAGAGACTTCAAGGAC	Locke et al., 2012
Control genes		
Actin	F: TGCCAACACTGTCCTTTCTG R: AGAATTGACCCACCAATCCA	Lourenço et al., 2008
Elongation factor 1	F: GGAGATGCTGCCATCGTTAT R: CAGCAGCGTCCTTGAAAGTT	Lourenço et al., 2008
Ribosomal protein S5	F: AATTATTTGGTCGCTGGAATTG R: TAACGTCCAGCAGAATGTGGTA	Evans, 2006

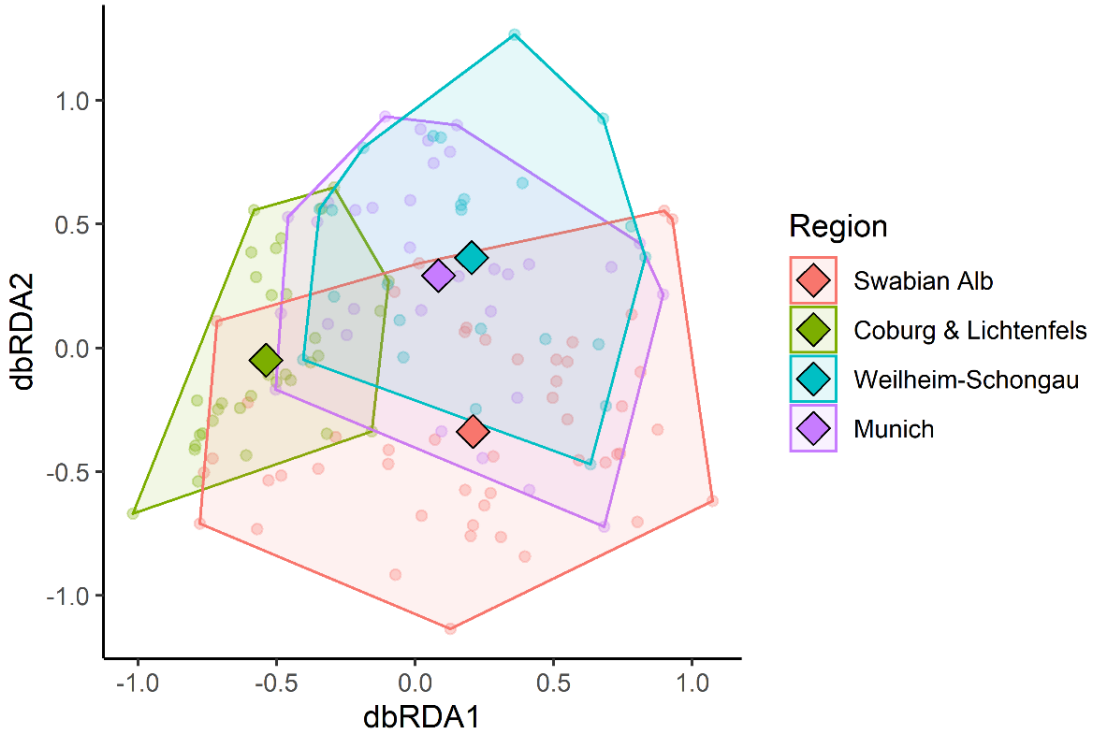

Figure S3.1: Dissimilarity of microparasite communities in honeybee colonies (N = 138) between the four study regions as revealed by a redundancy analysis with "region" as a constraining factor. Region of sampling explains 14.9% of variation in parasite community composition based on presence/absence of taxa (Jaccard distance). The first two constrained axes explain 6.5% (dbRDA1) and 5.2% (dbRDA2) of the variation. Dots are colony locations and diamonds are mean locations.

Table S3.2: Result of a likelihood ratio test (function "anova()" in R) comparing two nested models of the number of detected parasites. The factor "region" significantly improves model fit compared to a null model.

Model	Df	AIC	BIC	logLik	deviance	Chisq	Δ Df	Pr(>Chisq)
glmmTMB(Number of parasites ~ **1**, family = genpois())	2	487.20	493.05	-241.60	483.20	-	-	-
glmmTMB(Number of parasites ~ **region**, dispformula = ~region, family = genpois())	8	472.38	495.79	-228.19	456.38	26.82	6	0.00016

Table S3.3: Result of a likelihood ratio test (function "anova()" in R) comparing two nested models explaining the number of detected parasites. Adding the factor "management" (feral versus managed) to the factor "region" significantly improves model fit.

Model	Df	AIC	BIC	logLik	deviance	Chisq	Δ Df	Pr(>Chisq)
glmmTMB(Number of parasites ~ **region,** dispformula = ~region, family = genpois())	8	472.38	495.80	-228.19	456.38	-	-	-
glmmTMB(Number of parasites ~ **management + region**, dispformula = ~region, family = genpois())	9	459.70	486.05	-220.85	441.70	14.68	1	0.00013

Table S3.4: Result of a likelihood ratio test (function "anova()" in R) comparing two nested models of the number of detected parasites. The interaction between management and region does not significantly improve model fit.

Model	Df	AIC	BIC	logLik	deviance	Chisq	Δ Df	Pr(>Chisq)
glmmTMB(Number of parasites ~ **management + region**, dispformula = ~region, family = genpois())	9	459.70	486.05	-220.85	441.70	-	-	-
glmmTMB(Number of parasites ~ **management + region + management:region**, dispformula = ~region, family = genpois())	12	464.76	499.88	-220.38	440.76	0.95	3	0.814

Table S3.5: Mean numbers of detected parasites per colony and the respective 95% confidence limits (CI) estimated by a generalized linear model (model formula: "glmmTMB(Number of parasites ~ management + region + management : region, dispformula = ~ region, family = genpois()") for all cases (overall), for managed cases and for feral cases, and divided by study region.

Region	Management	Estimate	Lower CI	Upper CI
Overall	*Overall*	5.768	5.573	5.97
	Managed	6.164	5.895	6.446
	Feral	5.397	5.132	5.676
Swabian Alb	*Overall*	5.718	5.329	6.135
	Managed	5.951	5.444	6.506
	Feral	5.493	4.938	6.111
Coburg & Lichtenfels	*Overall*	4.946	4.642	5.271
	Managed	5.951	5.444	6.506
	Feral	4.605	4.204	5.045
Weilheim-Schongau	*Overall*	6.318	5.853	6.820
	Managed	6.775	6.113	7.508
	Feral	5.892	5.278	6.576
Munich	*Overall*	6.195	5.815	6.601
	Managed	6.741	6.214	7.313
	Feral	5.693	5.184	6.253

Table S3.6: Overview of prevalence and colony-level loads of 15 detected microparasites for A: all colonies tested, M: managed colonies only, and F: feral colonies only. Three other parasites assayed in this study (*Acarapis woodi*, invertebrate iridescent virus 6, and Kashmir bee virus) were not detected in any colony.

				Prevalence (%)			Log_{10} (n / 100 ng RNA+1)		
Parasite Taxon	**Sample**	**N**	**N (positives)**	**Estimate**	**Lower CI**	**Upper CI**	**Mean**	**Mean (positives)**	**Max**
Crithidia/ Lotmaria	A	138	117	84.8	77.7	90.1	5.99	7.06	8.84
	M	74	67	90.5	81.5	95.7	6.37	7.04	8.75
	F	64	50	78.1	66.6	87.2	5.54	7.09	8.84
Nosema apis	A	138	6	4.3	1.9	9.2	0.21	4.82	9.37
	M	74	3	4.1	1.1	11	0.13	3.19	3.49
	F	64	3	4.7	1.3	12.8	0.3	6.45	9.37
Nosema ceranae	A	138	133	96.4	91.9	98.6	6.55	6.80	9.59
	M	74	71	95.9	89	98.9	6.7	6.98	9.57
	F	64	62	96.9	89.6	99.4	6.39	6.593	9.59
Bacteria									
Melissococcus plutonius	A	138	11	8	4.2	13.6	0.22	2.75	3.71
	M	74	6	8.1	3.6	16.5	0.25	3.10	3.71
	F	64	5	7.8	3.1	16.8	0.18	2.32	3.22
Paenibacillus larvae	A	138	1	0.7	0	3.7	0.03	3.68	3.68
	M	74	1	1.4	0.1	7	0.05	3.68	3.68
	F	64	0	0	0	5.6	0	NA	0
Viruses									
Acute bee paralysis virus	A	138	69	50	41.6	58.4	2.71	5.42	8.12
	M	74	40	54.1	42.5	65.3	2.97	5.49	8.12
	F	64	29	45.3	33.4	57.9	2.41	5.31	7.90
Black queen cell virus	A	138	131	94.9	90.1	97.7	5.45	5.74	8.55
	M	74	70	94.6	86.9	98.1	5.51	5.83	8.55
	F	64	61	95.3	87.2	98.7	5.38	5.65	8.30
Chronic bee paralysis virus	A	138	12	8.7	4.8	14.6	0.53	6.09	7.93
	M	74	11	14.9	8.1	24.7	0.88	5.94	7.93
	F	64	1	1.6	0.1	8	0.12	7.71	7.71
Deformed wing virus A	A	138	16	11.6	7	17.9	0.58	4.99	9.28
	M	74	14	18.9	11	29.5	0.96	5.08	9.28
	F	64	2	3.1	0.6	10.4	0.14	4.36	4.45
Deformed wing virus B	A	138	58	42	34	50.6	2.63	6.27	8.63
	M	74	35	47.3	35.6	58.9	3.01	6.37	8.63
	F	64	23	35.9	24.6	48.4	2.2	6.11	8.41
Israeli acute paralysis virus	A	138	2	1.4	0.3	5.1	0.02	1.34	1.45
	M	74	0	0	0	4.8	0	NA	0
	F	64	2	3.1	0.6	10.4	0.04	1.34	1.45
Lake Sinai virus	A	138	127	92	86.4	95.8	5.31	5.77	7.58
	M	74	69	93.2	85.4	97.3	5.38	5.78	7.58
	F	64	58	90.6	80.9	95.8	5.22	5.77	7.41
Sacbrood virus	A	138	72	52.2	43.8	60.6	2.33	4.46	8.37
	M	74	40	54.1	42.5	65.3	2.42	4.47	8.37
	F	64	32	50	37.3	62.7	2.23	4.46	6.98
Slow bee paralysis virus	A	138	3	2.2	0.6	6.2	0.06	2.92	4.49
	M	74	2	2.7	0.5	9	0.09	3.49	4.49
	F	64	1	1.6	0.1	8	0.03	1.78	1.78
Varroa destructor macula-like virus	A	138	5	3.6	1.4	8.1	0.09	2.58	3.02
	M	74	3	4.1	1.1	11	0.11	2.63	3.02
	F	64	2	3.1	0.6	10.4	0.08	2.51	2.91

Table S3.7: Overview of prevalence and colony-level loads of 15 microparasites for study region 1 (Swabian Alb). Three other parasites assayed in this study (*Acarapis woodi*, invertebrate iridescent virus 6, and Kashmir bee virus) were not detected any of the four study regions. A: all colonies, M: managed colonies, and F: feral colonies.

				Prevalence (%)			Log_{10} (n / 100 ng RNA+1)		
Parasite Taxon	**Sample**	**N**	**N (positives)**	**Estimate**	**Lower CI**	**Upper CI**	**Mean**	**Mean (positives)**	**Max**
Crithidia/ Lotmaria	A	49	39	79.6	66.3	89.5	5.39	6.77	8.84
	M	28	25	89.3	72.2	97	6.02	6.75	8.48
	F	21	14	66.7	44.9	84.8	4.55	6.82	8.84
Nosema apis	A	49	6	12.2	5.5	24.1	0.59	4.82	9.37
	M	28	3	10.7	3	27.8	0.34	3.19	3.49
	F	21	3	14.3	4	35.1	0.92	6.45	9.37
Nosema ceranae	A	49	46	93.9	83.3	98.3	6.31	6.73	9.54
	M	28	26	92.9	77.6	98.7	6.4	6.89	9.54
	F	21	20	95.2	77.3	99.8	6.2	6.51	9.45
Bacteria									
Melissococcus plutonius	A	49	2	4.1	0.7	13.6	0.14	3.33	3.43
	M	28	1	3.6	0.2	17	0.12	3.43	3.43
	F	21	1	4.8	0.2	22.7	0.15	3.22	3.22
Paenibacillus larvae	A	49	1	2	0.1	10.5	0.08	3.68	3.68
	M	28	1	3.6	0.2	17	0.13	3.68	3.68
	F	21	0	0	0	15.2	0	NA	0
Viruses									
Acute bee paralysis virus	A	49	38	77.6	63.5	87.4	4.61	5.94	7.9
	M	28	20	71.4	51.8	85.8	3.96	5.54	7.61
	F	21	18	85.7	64.9	96	5.47	6.39	7.9
Black queen cell virus	A	49	44	89.8	78.1	95.9	4.9	5.46	7.71
	M	28	25	89.3	72.2	97	4.89	5.48	7.71
	F	21	19	90.5	69.9	98.3	4.92	5.44	7.52
Chronic bee paralysis virus	A	49	3	6.1	1.7	16.7	0.31	5.06	7.9
	M	28	3	10.7	3	27.8	0.54	5.06	7.9
	F	21	0	0	0	15.2	0	NA	0
Deformed wing virus A	A	49	5	10.2	4.1	21.9	0.45	4.38	4.57
	M	28	4	14.3	5	31.6	0.63	4.41	4.57
	F	21	1	4.8	0.2	22.7	0.2	4.27	4.27
Deformed wing virus B	A	49	10	20.4	10.5	33.7	1.41	6.92	8.42
	M	28	7	25	11.4	44.5	1.67	6.67	8.42
	F	21	3	14.3	4	35.1	1.07	7.5	7.91
Israeli acute paralysis virus	A	49	2	4.1	0.7	13.6	0.05	1.34	1.45
	M	28	0	0	0	11.4	0	NA	0
	F	21	2	9.5	1.7	30.1	0.13	1.34	1.45
Lake Sinai virus	A	49	46	93.9	83.3	98.3	5.24	5.59	7.58
	M	28	28	100	88.6	100	5.73	5.73	7.58
	F	21	18	85.7	64.9	96	4.59	5.36	6.98
Sacbrood virus	A	49	27	55.1	40.6	68.7	2.3	4.18	6.06
	M	28	18	64.3	44.5	80.8	2.73	4.24	6.06
	F	21	9	42.9	22.7	64.9	1.73	4.04	5.57
Slow bee paralysis virus	A	49	3	6.1	1.7	16.7	0.18	2.92	4.49
	M	28	2	7.1	1.3	22.4	0.25	3.49	4.49
	F	21	1	4.8	0.2	22.7	0.08	1.78	1.78
Varroa destructor macula-like virus	A	49	1	2	0.1	10.5	0.05	2.28	2.28
	M	28	1	3.6	0.2	17	0.08	2.28	2.28
	F	21	0	0	0	15.2	0	NA	0

Table S3.8: Overview of prevalence and colony-level loads of 15 microparasites for study region 2 (Coburg & Lichtenfels). Three other parasites assayed in this study (*Acarapis woodi*, invertebrate iridescent virus 6, and Kashmir bee virus) were not detected in any of the four study regions. A: all colonies, M: managed colonies, and F: feral colonies.

				Prevalence (%)			Log_{10} (n / 100 ng RNA+1)		
Parasite Taxon	**Sample**	**N**	**N (positives)**	**Estimate**	**Lower CI**	**Upper CI**	**Mean**	**Mean (positives)**	**Max**
Crithidia/ Lotmaria	A	33	28	84.8	68.7	93.8	5.92	6.98	8.59
	M	17	15	88.2	65.4	97.9	6.24	7.07	8.59
	F	16	13	81.3	56.6	94.7	5.59	6.88	8.51
Nosema apis	A	33	0	0	0	9.6	0	NA	0
	M	17	0	0	0	18.9	0	NA	0
	F	16	0	0	0	20.1	0	NA	0
Nosema ceranae	A	33	33	100	90.4	100	8.28	8.28	9.59
	M	17	17	100	81.1	100	8.65	8.65	9.51
	F	16	16	100	79.9	100	7.88	7.88	9.59
Bacteria									
Melissococcus plutonius	A	33	3	9.1	2.5	23.6	0.21	2.27	2.48
	M	17	0	0	0	18.9	0	NA	0
	F	16	3	18.8	5.3	43.4	0.43	2.27	2.48
Paenibacillus larvae	A	33	0	0	0	9.6	0	NA	0
	M	17	0	0	0	18.9	0	NA	0
	F	16	0	0	0	20.1	0	NA	0
Viruses									
Acute bee paralysis virus	A	33	2	6.1	1.1	19.2	0.15	2.47	2.69
	M	17	2	11.8	2.1	34.6	0.29	2.47	2.69
	F	16	0	0	0	20.1	0	NA	0
Black queen cell virus	A	33	33	100	90.4	100	5.35	5.35	8.13
	M	17	17	100	81.1	100	5.28	5.28	8.13
	F	16	16	100	79.9	100	5.43	5.43	7.31
Chronic bee paralysis virus	A	33	4	12.1	4.2	28.2	0.75	6.21	7.49
	M	17	4	23.5	8.5	48.9	1.46	6.21	7.49
	F	16	0	0	0	20.1	0	NA	0
Deformed wing virus A	A	33	5	15.2	6.2	31.3	0.7	4.61	4.9
	M	17	5	29.4	12.4	54.4	1.36	4.61	4.9
	F	16	0	0	0	20.1	0	NA	0
Deformed wing virus B	A	33	10	30.3	15.7	48.5	1.48	4.89	7
	M	17	6	35.3	16.4	59.4	1.69	4.8	7
	F	16	4	25	9	50	1.26	5.03	5.57
Israeli acute paralysis virus	A	33	0	0	0	9.6	0	NA	0
	M	17	0	0	0	18.9	0	NA	0
	F	16	0	0	0	20.1	0	NA	0
Lake Sinai virus	A	33	31	93.9	80.8	98.9	5.55	5.91	7.34
	M	17	15	88.2	65.4	97.9	5.1	5.78	7.18
	F	16	16	100	79.9	100	6.03	6.03	7.34
Sacbrood virus	A	33	11	33.3	19	51.6	1.66	4.98	6.98
	M	17	4	23.5	8.5	48.9	1.1	4.68	5.83
	F	16	7	43.8	20.1	70	2.25	5.15	6.98
Slow bee paralysis virus	A	33	0	0	0	9.6	0	NA	0
	M	17	0	0	0	18.9	0	NA	0
	F	16	0	0	0	20.1	0	NA	0
Varroa destructor macula-like virus	A	33	0	0	0	9.6	0	NA	0
	M	17	0	0	0	18.9	0	NA	0
	F	16	0	0	0	20.1	0	NA	0

Table S3.9: Overview of prevalence and colony-level loads of 15 microparasites for study region 3 (Weilheim-Schongau). Three other parasites assayed in this study (*Acarapis woodi*, invertebrate iridescent virus 6, and Kashmir bee virus) were not detected in any of the four study regions. A: all colonies, M: managed colonies, and F: feral colonies.

				Prevalence (%)			Log_{10} (n / 100 ng RNA+1)		
Parasite Taxon	**Sample**	**N**	**N (positives)**	**Estimate**	**Lower CI**	**Upper CI**	**Mean**	**Mean (positives)**	**Max**
Crithidia/ Lotmaria	A	24	24	100	86.7	100	7.73	7.73	8.75
	M	12	12	100	76.4	100	7.83	7.83	8.75
	F	12	12	100	76.4	100	7.62	7.62	8.55
Nosema apis	A	24	0	0	0	13.3	0	NA	0
	M	12	0	0	0	23.6	0	NA	0
	F	12	0	0	0	23.6	0	NA	0
Nosema ceranae	A	24	22	91.7	73.8	98.5	5.25	5.73	9.35
	M	12	11	91.7	63.4	99.6	5.47	5.96	9.35
	F	12	11	91.7	63.4	99.6	5.04	5.49	8.85
Bacteria									
Melissococcus plutonius	A	24	5	20.8	8.6	41.4	0.56	2.68	3.71
	M	12	4	33.3	12.3	63.4	0.99	2.96	3.71
	F	12	1	8.3	0.4	36.6	0.13	1.58	1.58
Paenibacillus larvae	A	24	0	0	0	13.3	0	NA	0
	M	12	0	0	0	23.6	0	NA	0
	F	12	0	0	0	23.6	0	NA	0
Viruses									
Acute bee paralysis virus	A	24	14	58.3	37	76.6	2.47	4.24	8.12
	M	12	9	75	45.6	92.8	3.95	5.26	8.12
	F	12	5	41.7	18.1	70.6	1	2.41	2.8
Black queen cell virus	A	24	23	95.8	80.2	99.8	5.84	6.09	8.55
	M	12	12	100	76.4	100	6.27	6.27	8.55
	F	12	11	91.7	63.4	99.6	5.4	5.89	8.3
Chronic bee paralysis virus	A	24	0	0	0	13.3	0	NA	0
	M	12	0	0	0	23.6	0	NA	0
	F	12	0	0	0	23.6	0	NA	0
Deformed wing virus A	A	24	0	0	0	13.3	0	NA	0
	M	12	0	0	0	23.6	0	NA	0
	F	12	0	0	0	23.6	0	NA	0
Deformed wing virus B	A	24	14	58.3	37	76.6	3.6	6.17	8.45
	M	12	8	66.7	36.6	87.7	4.39	6.58	8.45
	F	12	6	50	23.4	76.6	2.81	5.62	8.41
Israeli acute paralysis virus	A	24	0	0	0	13.3	0	NA	0
	M	12	0	0	0	23.6	0	NA	0
	F	12	0	0	0	23.6	0	NA	0
Lake Sinai virus	A	24	18	75	54.3	88.5	3.59	4.79	6.79
	M	12	9	75	45.6	92.8	3.34	4.45	6.77
	F	12	9	75	45.6	92.8	3.84	5.12	6.79
Sacbrood virus	A	24	13	54.2	33.9	73.8	2.44	4.5	6.11
	M	12	8	66.7	36.6	87.7	3.11	4.67	5.59
	F	12	5	41.7	18.1	70.6	1.76	4.22	6.11
Slow bee paralysis virus	A	24	0	0	0	13.3	0	NA	0
	M	12	0	0	0	23.6	0	NA	0
	F	12	0	0	0	23.6	0	NA	0
Varroa destructor macula-like virus	A	24	0	0	0	13.3	0	NA	0
	M	12	0	0	0	23.6	0	NA	0
	F	12	0	0	0	23.6	0	NA	0

Table S3.10: Overview of prevalence and colony-level loads of 15 microparasites for study region 4 (Munich). Three other parasites assayed in this study (*Acarapis woodi*, invertebrate iridescent virus 6, and Kashmir bee virus) were not detected in any of the four study regions. A: all colonies, M: managed colonies, and F: feral colonies.

				Prevalence (%)			Log_{10} (n / 100 ng RNA+1)		
Parasite Taxon	**Sample**	**N**	**N (positives)**	**Estimate**	**Lower CI**	**Upper CI**	**Mean**	**Mean (positives)**	**Max**
Crithidia/ Lotmaria	A	32	26	81.3	64.4	91.5	5.66	6.97	8.49
	M	17	15	88.2	65.4	97.9	6.06	6.86	8.49
	F	15	11	73.3	46.5	90.3	5.22	7.12	8.14
Nosema apis	A	32	0	0	0	9.9	0	NA	0
	M	17	0	0	0	18.9	0	NA	0
	F	15	0	0	0	21.5	0	NA	0
Nosema ceranae	A	32	32	100	90.1	100	6.12	6.12	9.57
	M	17	17	100	81.1	100	6.12	6.12	9.57
	F	15	15	100	78.5	100	6.13	6.13	9.35
Bacteria									
Melissococcus plutonius	A	32	1	3.1	0.2	16.2	0.1	3.34	3.34
	M	17	1	5.9	0.3	28.2	0.2	3.34	3.34
	F	15	0	0	0	21.5	0	NA	0
Paenibacillus larvae	A	32	0	0	0	9.9	0	NA	0
	M	17	0	0	0	18.9	0	NA	0
	F	15	0	0	0	21.5	0	NA	0
Viruses									
Acute bee paralysis virus	A	32	15	46.9	29.5	64.4	2.61	5.57	7.81
	M	17	9	52.9	28.2	74.7	3.32	6.28	7.81
	F	15	6	40	18.6	66.8	1.8	4.5	7.64
Black queen cell virus	A	32	31	96.9	83.8	99.8	6.11	6.3	7.93
	M	17	16	94.1	71.8	99.7	6.23	6.62	7.88
	F	15	15	100	78.5	100	5.97	5.97	7.93
Chronic bee paralysis virus	A	32	5	15.6	6.4	32.3	1.03	6.6	7.93
	M	17	4	23.5	8.5	48.9	1.49	6.32	7.93
	F	15	1	6.7	0.3	30.2	0.51	7.71	7.71
Deformed wing virus A	A	32	6	18.8	8.5	35.6	1.09	5.82	9.28
	M	17	5	29.4	12.4	54.4	1.79	6.1	9.28
	F	15	1	6.7	0.3	30.2	0.3	4.45	4.45
Deformed wing virus B	A	32	24	75	57.7	87.8	4.97	6.62	8.63
	M	17	14	82.4	58.3	95	5.57	6.76	8.63
	F	15	10	66.7	39.4	85.8	4.29	6.43	8.27
Israeli acute paralysis virus	A	32	0	0	0	9.9	0	NA	0
	M	17	0	0	0	18.9	0	NA	0
	F	15	0	0	0	21.5	0	NA	0
Lake Sinai virus	A	32	32	100	90.1	100	6.46	6.46	7.48
	M	17	17	100	81.1	100	6.55	6.55	7.48
	F	15	15	100	78.5	100	6.36	6.36	7.41
Sacbrood virus	A	32	21	65.6	47.3	80.4	2.98	4.55	8.36
	M	17	10	58.8	34.6	81.1	2.72	4.63	8.36
	F	15	11	73.3	46.5	90.3	3.28	4.47	5.95
Slow bee paralysis virus	A	32	0	0	0	9.9	0	NA	0
	M	17	0	0	0	18.9	0	NA	0
	F	15	0	0	0	21.5	0	NA	0
Varroa destructor macula-like virus	A	32	4	12.5	4.4	28.2	0.33	2.66	3.02
	M	17	2	11.8	2.1	34.6	0.33	2.81	3.02
	F	15	2	13.3	2.4	39.4	0.33	2.51	2.91

Table S3.11: Result of a likelihood ratio test (function "anova()" in R) comparing two nested models explaining the number of detected parasites. Adding the factor "colony type" to the factor "region" significantly improves model fit.

Model	Df	AIC	BIC	logLik	deviance	Chisq	Δ Df	Pr(>Chisq)
glmmTMB(Number of parasites ~ **region**, dispformula = ~region, family = genpois()	8	472.38	495.80	-228.19	456.38	-	-	-
glmmTMB(Number of parasites ~ **colony type + region**, dispformula = ~region, family = genpois()	12	457.15	492.28	-216.58	433.15	23.23	4	0.0001

Table S3.12: Result of a likelihood ratio test (function "anova()" in R) comparing two nested models of the number of detected parasites. The interaction between "colony type" and "region" does not significantly improve model fit.

Model	Df	AIC	BIC	logLik	deviance	Chisq	Δ Df	Pr(>Chisq)
glmmTMB(Number of parasites ~ **colony type + region**, dispformula = ~region, family = genpois())	12	457.15	492.28	-216.58	433.15			
glmmTMB(Number of parasites ~ **colony type + region + colony type:region**, dispformula = ~region, family = genpois())	24	471.60	541.86	-211.80	423.60	9.55	12	0.656

Table S3.13: Mean numbers of detected parasites per colony and the respective 95% confidence limits (CI) estimated by a generalized linear model (model formula: glmmTMB(Number of parasites ~ colony type + region + colony type:region, dispformula = ~ region, family = genpois()) for the five colony types and divided by study region.

Region	Colony type	Estimate	Lower CI	Upper CI
Swabian Alb	Managed overwintered	6.052	5.373	6.817
	Managed hived swarm	7.273	5.412	9.773
	Managed nucleus	5.587	4.875	6.403
	Feral overwintered	5.875	4.672	7.388
	Feral founder	5.423	4.836	6.080
Coburg & Lichtenfels	Managed overwintered	5.576	5.092	6.106
	Managed hived swarm	5.978	5.126	6.972
	Managed nucleus	4.280	3.718	4.928
	Feral overwintered	4.577	3.916	5.350
	Feral founder	4.681	4.290	5.108
Weilheim-Schongau	Managed overwintered	6.907	6.052	7.883
	Managed hived swarm	6.931	5.526	8.692
	Managed nucleus	6.529	5.516	7.728
	Feral overwintered	6.435	5.618	7.372
	Feral founder	5.328	4.603	6.168
Munich	Managed overwintered	6.956	6.253	7.738
	Managed hived swarm	6.461	5.177	8.065
	Managed nucleus	6.503	5.682	7.443
	Feral overwintered	5.857	4.912	6.983
	Feral founder	5.639	5.063	6.280

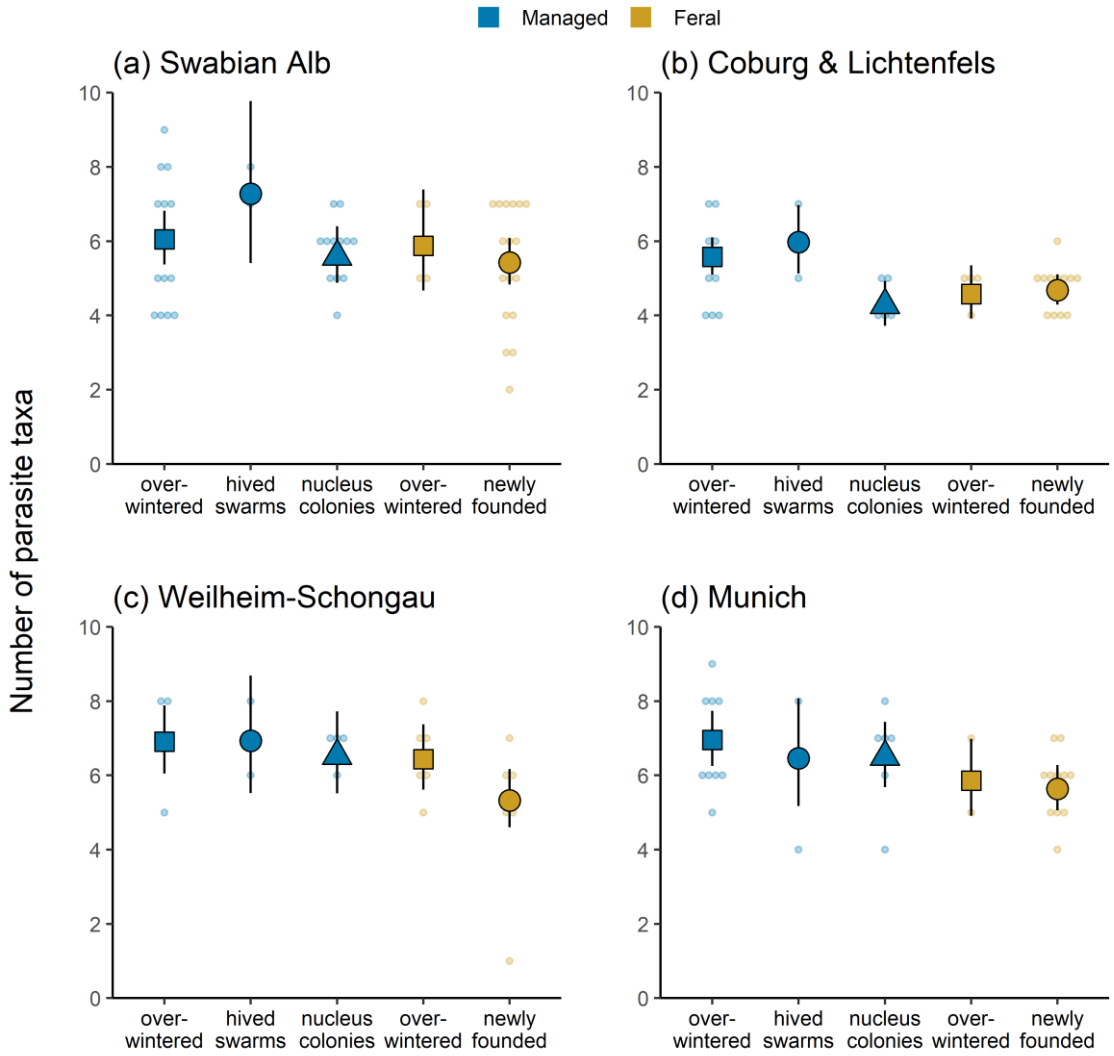

Figure S3.2 (a)–(d): Number of microparasite taxa detected among the 18 taxa assayed in relation to colony type for each of the four study regions. Dots are raw data; large symbols and vertical lines give model-estimated means and 95%-confidence intervals.

Table S3.14: Overview of prevalence and colony-level loads of 15 microparasites for five colony types. Three other parasites assayed in this study (*Acarapis woodi*, invertebrate iridescent virus 6, and Kashmir bee virus) were not detected in any colony. MO: overwintered managed colonies, MS: managed hived swarms, MN: managed nucleus colonies, FO: overwintered feral colonies and FF: newly founded feral colonies.

				Prevalence (%)			Log_{10} (n / 100 ng RNA+1)		
Parasite Taxon	**Colony type**	**N**	**N (positives)**	**Estimate**	**Lower CI**	**Upper CI**	**Mean**	**Mean (positives)**	**Max**
Crithidia/ Lotmaria	MO	38	36	94.7	82.4	99.1	6.92	7.31	8.75
	MS	9	7	77.8	44	95.9	6.15	7.91	8.59
	MN	27	24	88.9	71.1	96.9	5.68	6.39	8.39
	FO	18	14	77.8	52.9	92	5.92	7.61	8.35
	FF	46	36	78.3	64.4	88.8	5.4	6.89	8.84
Nosema apis	MO	38	2	5.3	0.9	17.6	0.16	3.04	3.2
	MS	9	1	11.1	0.6	44.3	0.39	3.49	3.49
	MN	27	0	0	0	11.8	0	NA	0
	FO	18	1	5.6	0.3	26.6	0.16	2.94	2.94
	FF	46	2	4.3	0.8	14.5	0.36	8.21	9.37
Nosema ceranae	MO	38	37	97.4	86.4	99.9	7.06	7.26	9.57
	MS	9	9	100	68.4	100	7.51	7.51	9.54
	MN	27	25	92.6	76.7	98.7	5.92	6.39	9.51
	FO	18	18	100	82.2	100	6.58	6.58	9.51
	FF	46	44	95.7	85.5	99.2	6.31	6.6	9.59
Bacteria									
Melissococcus plutonius	MO	38	4	10.5	3.7	24.4	0.35	3.33	3.71
	MS	9	1	11.1	0.6	44.3	0.26	2.3	2.3
	MN	27	1	3.7	0.2	17.6	0.11	2.96	2.96
	FO	18	1	5.6	0.3	26.6	0.09	1.58	1.58
	FF	46	4	8.7	3	20.1	0.22	2.51	3.22
Paenibacillus larvae	MO	38	1	2.6	0.1	13.6	0.1	3.68	3.68
	MS	9	0	0	0	31.6	0	NA	0
	MN	27	0	0	0	11.8	0	NA	0
	FO	18	0	0	0	17.8	0	NA	0
	FF	46	0	0	0	6.9	0	NA	0
Viruses									
Acute bee paralysis virus	MO	38	19	50	33.8	66.2	2.83	5.66	7.61
	MS	9	6	66.7	31.6	90.2	3.52	5.29	8.12
	MN	27	15	55.6	36.6	73.1	2.98	5.36	7.81
	FO	18	9	50	26.6	73.4	2.52	5.03	7.9
	FF	46	20	43.5	29.2	58.9	2.36	5.44	7.87
Black queen cell virus	MO	38	34	89.5	75.6	96.3	5.18	5.79	7.88
	MS	9	9	100	68.4	100	5.56	5.56	8.05
	MN	27	27	100	88.2	100	5.96	5.96	8.55
	FO	18	18	100	82.2	100	5.84	5.84	8.3
	FF	46	43	93.5	82.2	98.2	5.21	5.57	7.93
Chronic bee paralysis virus	MO	38	7	18.4	8.4	33.8	1.26	6.86	7.93
	MS	9	3	33.3	9.8	68.4	1.4	4.2	7.33
	MN	27	1	3.7	0.2	17.6	0.17	4.66	4.66
	FO	18	1	5.6	0.3	26.6	0.43	7.71	7.71
	FF	46	0	0	0	6.9	0	NA	0
Deformed wing virus A	MO	38	8	21.1	10	36.5	1.15	5.45	9.28
	MS	9	1	11.1	0.6	44.3	0.54	4.9	4.9
	MN	27	5	18.5	7.6	36.7	0.84	4.53	4.65
	FO	18	0	0	0	17.8	0	NA	0
	FF	46	2	4.3	0.8	14.5	0.19	4.36	4.45

Table S3.14 (*continued*).

Parasite Taxon	Colony type	N	N (positives)	Prevalence (%) Estimate	Lower CI	Upper CI	Log_{10} (n / 100 ng RNA+1) Mean	Mean (positives)	Max
Deformed wing virus B	MO	38	20	52.6	36.5	68.9	3.32	6.31	8.63
	MS	9	5	55.6	25.1	83.1	3.5	6.3	7.96
	MN	27	10	37	20.2	57.1	2.41	6.52	8.45
	FO	18	11	61.1	37.5	82.2	3.68	6.02	7.91
	FF	46	12	26.1	14.5	41.1	1.62	6.2	8.41
Israeli acute paralysis virus	MO	38	0	0	0	8.4	0	NA	0
	MS	9	0	0	0	31.6	0	NA	0
	MN	27	0	0	0	11.8	0	NA	0
	FO	18	0	0	0	17.8	0	NA	0
	FF	46	2	4.3	0.8	14.5	0.06	1.34	1.45
Lake Sinai virus	MO	38	36	94.7	82.4	99.1	5.34	5.64	7.58
	MS	9	8	88.9	55.7	99.4	5.52	6.21	7.07
	MN	27	25	92.6	76.7	98.7	5.4	5.83	7.39
	FO	18	17	94.4	73.4	99.7	5.95	6.3	7.31
	FF	46	41	89.1	76.6	95.6	4.94	5.54	7.41
Sacbrood virus	MO	38	19	50	33.8	66.2	2.19	4.38	6.99
	MS	9	4	44.4	16.9	74.9	1.73	3.88	4.48
	MN	27	17	63	42.9	79.8	2.96	4.71	8.36
	FO	18	8	44.4	23.6	67.4	2	4.5	6.11
	FF	46	24	52.2	37.8	66.6	2.32	4.44	6.98
Slow bee paralysis virus	MO	38	2	5.3	0.9	17.6	0.18	3.49	4.49
	MS	9	0	0	0	31.6	0	NA	0
	MN	27	0	0	0	11.8	0	NA	0
	FO	18	0	0	0	17.8	0	NA	0
	FF	46	1	2.2	0.1	11.2	0.04	1.78	1.78
Varroa destructor macula-like virus	MO	38	2	5.3	0.9	17.6	0.15	2.81	3.02
	MS	9	0	0	0	31.6	0	NA	0
	MN	27	1	3.7	0.2	17.6	0.08	2.28	2.28
	FO	18	1	5.6	0.3	26.6	0.12	2.11	2.11
	FF	46	1	2.2	0.1	11.2	0.06	2.91	2.91

Chapter four

Parasites, depredators and limited resources as potential drivers of winter mortality of feral honeybee colonies in German forests

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Abstract

Wild honeybees (*Apis mellifera*) are considered extinct in most parts of Europe. The likely causes of their decline include increased parasite burden, lack of high-quality nesting sites and associated depredation pressure, and food scarcity. In Germany, feral honeybees still colonise managed forests, but their survival rate is too low to maintain viable populations. Based on colony observations collected during a monitoring study, data on parasite prevalence, experiments on nest depredation, and analyses of land cover maps, we explored whether parasite pressure, depredation or expected landscape-level food availability explain feral colony winter mortality. Considering the colony-level occurrence of 18 microparasites in the previous summer, colonies that died did not have a higher parasite burden than colonies that survived. Camera traps installed at cavity trees revealed that four woodpecker species, great tits, and pine martens act as nest depredators. In a depredator exclusion experiment, the winter survival rate of colonies in cavities with protected entrances was 50% higher than that of colonies with unmanipulated entrances. Landscapes surrounding surviving colonies contained on average 6.4 percentage points more cropland than landscapes surrounding dying colonies, with cropland being known to disproportionately provide forage for bees in our study system. We conclude that the lack of spacious but well-protected nesting cavities and the shortage of food are currently more important than parasites in limiting populations of wild-living honeybees in German forests. Increasing the density and diversity of large tree cavities and promoting bee forage plants in forests will probably promote wild-living honeybees despite parasite pressure.

Introduction

Europe is experiencing accelerated declines of its insect populations, calling for research to identify the drivers (Habel, Samways & Schmitt, 2019; Wagner, 2020). In contrast to the negative trends that hold for many taxa, one of the ecologically and economically most important species, the western honeybee, seems to be less affected. Even in mid-latitude countries, the number of managed honeybee colonies increased during the last decade (FAO 2022), a trend aligning with the long-term growth of managed honeybee populations worldwide (Moritz & Erler, 2016; Herrera, 2020; Phiri, Fèvre & Hidano, 2022). However, *Apis mellifera* also exists in the wild, and wild populations do not necessarily mirror managed population increases since the former can be limited by factors not relevant under human management. Wild-living colonies outnumber managed hives in Africa and in parts of the species' introduced range (Jaffé et al., 2010; Pirk, Crewe & Moritz, 2017; Visick & Ratnieks, 2022), but it is assumed that self-sustaining populations have gone extinct in most parts of Europe (Pirk, Crewe & Moritz, 2017; Requier et al., 2019a). Unfortunately, long-term data are lacking, and the drivers of wild honeybee declines have not been investigated (Kohl & Rutschmann, 2018).

Within the last decade, targeted censuses have revealed that wild-living colonies can still be found in various European countries (Oleksa, Gawroński & Tofilski, 2013; Fontana et al., 2018; Kohl & Rutschmann, 2018; Browne et al., 2021; Dubaić et al., 2021; Oberreiter et al., 2021; Rutschmann et al., 2022). However, this does not prove the existence of viable populations. For example, a demographic study of wild-living honeybee populations in managed forests in Germany showed that these are far from being self-sustaining (Kohl, Rutschmann & Steffan-Dewenter, 2022). Each spring, tree cavities are colonised by feral swarms that escaped from apiaries, but a high winter colony mortality prevents population establishment. Answering the question of why the survival of feral honeybees is currently hampered can also provide insights into the causes of historical wild honeybee population declines. This, in turn, is relevant for nature conservation more generally because the diverse natural habitat requirements of honeybees (e.g., the presence of large tree cavities and the supply of floral resources) overlap with those of many other species.

It is commonly assumed that the decline of wild honeybees was caused by the ectoparasitic mite *Varroa destructor*, which invaded Europe in the 1970s (Thompson et al., 2014; Meixner, Kryger & Costa, 2015). However, this is an indirect inference based on the experience that colonies managed in apiaries usually die within a few years when they are not treated against the parasite (Rosenkranz, Aumeier & Ziegelmann, 2010). While the mite certainly represents a threat to any population of naïve honeybees, there are indications that European wild honeybee populations were already extinct before the arrival of the new virus vector. For example, in his monograph on

the bee fauna of Franconia from 1933, Stoeckhert already stated that wild honeybees have disappeared from their natural habitat. As the leading cause, he identified the lack of tree cavities in forests managed for timber (Stoeckhert, 1933).

The availability of high-quality nesting cavities is probably an important limiting factor for wild honeybees under natural conditions (Ruttner, 1988a; Seeley, 2010), and since managed forests provide much lower densities of cavities than natural forests, the lack of cavities is probably even more severe today (Remm & Lõhmus, 2011; Courbaud et al., 2022). The only large tree cavities (>10 L volume) that are regularly found in central European managed forests are those excavated by the black woodpecker (*Dryocopus martius*). However, there is a high competition for nest sites among a range of secondary cavity-nesting species (Johnson, Nilsson & Tjernberg, 1993; Kosiński et al., 2010; Sikora, Schnitt & Kinser, 2016; Zahner, Bauer & Kaphegyi, 2017). For honeybee colonies that have successfully occupied a woodpecker cavity, competitive and/or antagonistic interactions at nest sites might therefore represent an additional challenge. During spring censuses of feral colonies, we regularly found pieces of beeswax comb on the forest floor beneath the cavity trees, suggesting that nest depredation had occurred. However, the question is whether cavity intruders are responsible for the bees' death or whether they merely take over the cavities after the bees have passed away.

While parasite pressure and winter nest depredation are specific threats to honeybees, a key factor limiting bees and many other animal populations is food availability (White, 2008; Scheper et al., 2014; Carvell et al., 2017; Ganser, Albrecht & Knop, 2021; Parreño et al., 2022). Nectar limitation is largely buffered under apicultural management because beekeepers provide sugar solution outside the main nectar flows, but for wild-living honeybees, gathering enough nectar and pollen to build up the worker population and the honey stores needed to survive the winter is a major challenge (Seeley, 2019). The positive correlation between the probability of winter survival of wild-living colonies and the amount of flower-rich semi-natural habitat in the surroundings, as observed in an agricultural landscape in NW Spain (Rutschmann et al., 2022), suggests that food availability is an important limiting factor for wild-living honeybees. The colonies living in German forests might be especially prone to starvation since management practices have created dense forest stands dominated by a few tree species which – apart from seasonal pulses of honeydew secreted by tree-sucking insects – provide little bee forage compared to open habitats (Rutschmann, Kohl & Steffan-Dewenter, 2023).

The known populations of feral honeybee colonies in German forests can be used to explore whether parasite burden, nest depredation or landscape context are associated with winter survival. Under the hypothesis that parasites are currently limiting feral colony winter mortality, the prediction is that colonies that die are infested with higher numbers of parasite taxa or different

parasite communities or suffer from higher colony-level parasite abundances than colonies that survive. For nest depredation to be a potential limiting factor, the prediction is that other animals enter honeybee nest cavities during winter and that colonies protected against intruders have a higher winter survival rate than colonies without protection. Knowing that major land cover types differ in the density of forage available for honeybees, the prediction of the forage limitation hypothesis is that surviving colonies are surrounded by landscapes with a higher proportion of flower-rich land cover than dying colonies. Here we use observations of feral colony overwintering, associated data on colony-level parasite burden, nest cavity observations, depredator exclusion experiments and landscape analyses to test these predictions.

Material and Methods

Study regions and feral honeybee colonies

We considered observations of winter mortality/survival of feral honeybee colonies inhabiting managed forests dominated by beech (*Fagus silvatica*) or spruce (*Picea abies*) in three regions in southern Germany: the Swabian Alb (centre of study region: N 48.34, E 9.48), the counties Coburg and Lichtenfels (N 50.25, E 10.96), and the county Weilheim-Schongau (N 47.85, E 10.87). The survival data were gathered between 2017 and 2021 during a monitoring study that investigated the population demography of wild-living honeybees (Kohl, Rutschmann & Steffan-Dewenter, 2022). The colonies were found by making systematic inspections of cavity trees that had been mapped before as part of regional strategies of forest nature conservation (Sikora, 2009) or in connection with periodical surveys of Nature 2000 areas of the Bavarian forest department. Most colonies (>98%) nested in cavities in beech trees made by the black woodpecker (*Dryocopus martius*), which comprise the largest source of potential homes for honeybees in German managed forests (Kohl & Rutschmann, 2018), and some colonies nested in other tree cavities in linden, spruce, or oak trees. We defined “winter” as the period between late September and the beginning of April. A total of 113 colony winter survival/mortality events involving 103 unique honeybee colonies and 71 different cavities were available. Depending on the type of analysis and the availability of associated data, we either considered all overwintering observations or a subset.

Parasite burden

To investigate whether diseases caused by parasites might be responsible for the high winter mortality of feral colonies, we tested whether there was an association between colony-level parasite burden in summer and the subsequent outcome of overwintering. We used data on the colony-level occurrence of 18 microparasites (covering eukaryotes, bacteria, and viruses) obtained using qPCR in a study comparing parasite burden between feral and managed honeybee colonies (Kohl et al., 2022). Besides 49 colony samples that had also been used in the original

study, we included data from another four colonies sampled in 2020 (these were not considered in the original study because colony age, which was a factor in the analysis, was unknown), and from 19 additional colonies collected in July 2019, totalling 67 combinations of colony-level parasite data and overwintering outcome. The parasite communities were analysed with the same method and in the same laboratory sessions as described in D'Alvise et al. (2019) and Kohl et al. (2022) (see chapter three). In brief, 20 bees per colony were collected at the nest entrance and total RNA was extracted from one multi-bee homogenate per colony using a TRIzol protocol. Colony-level parasite occurrence and abundance were determined from cDNA via high-throughput qPCR on a Biomark HD system (Standard BioTools, San Francisco, CA) using published primers for 18 microparasites (see supplementary information in chapter three for a list of parasites and control genes assayed). We considered as measures of parasite burden the colony-level prevalence (presence/absence) and the colony-level loads of each parasite taxon. Parasite loads were defined as the log of the number of target molecules per 100 ng of extracted RNA.

Nest depredation

To assess whether wild-living honeybee colonies are visited by other animals during winter and, if so, which species potentially act as nest depredators, we monitored feral colony nest entrances using camera traps (Zahner, Bauer & Kaphegyi, 2017). A total of fifteen cameras (Cuddeback Attack/Attack IR) were operated on different bee trees in two study regions, the Swabian Alb and the counties Coburg and Lichtenfels, between September 2019 and April 2020. We ascended trees using either a rope-climbing or a "trunk-climbing" technique (see supplementary information, Figure S4.1) and fixed the camera traps at 1.5–2 m above the cavity entrances using tension belts. The cameras were programmed to take one picture and one ten-second video upon motion detection, but we restricted recordings to one capture per 30 s to save battery power. A custom-built sledge system (Zahner, Bauer & Kaphegyi, 2017) allowed us to move the cameras up and down for inspections (Figure 4.1a). We checked the cameras every 6–8 weeks for data transfer from SD cards and battery changes. Due to our time-restricted recording scheme and the failures of some cameras during parts of the observation periods (due to damage by rainwater or wildlife, or quick battery exhaustion due to a high rate of false positive captures), we obtained complete records of cavity interactions for nine of the monitored cavities.

To directly test whether nest depredation negatively affects feral colony winter survival, we conducted depredator exclusion experiments. We protected cavity entrances with screens of wire mesh (mesh size: 8 mm) which we fixed to the tree trunk using a staple gun. The meshes excluded predators but allowed the passage of bees (Figure 4.1b). The experiments were performed in the same two regions in which camera traps were mounted and in two subsequent winters. During winter 2019/20 we protected 12 colonies with meshes and left 20 nests open as controls, and

during winter 2020/21 we had 20 mesh-protected nests and 20 control nests, totalling N = 32 treatments and N = 40 controls. Several cavity trees were considered in both the winters of 2019/20 and 2020/21. In these cases, we alternated the treatment, so the comparison was not biased by the over- or underrepresentation of any cavity.

Figure 4.1: Methods to investigate nest depredation of feral honeybee colonies nesting in black woodpecker cavities**. (a)** Camera trap mounted approx. 1.5 m above the nest entrance. **(b)** Cavity nest entrance protected by wire mesh to exclude nest predators.

Landscape context

To explore whether the availability of bee forage at the landscape scale potentially limits colony winter survival, we compared the composition of the landscapes surrounding colonies that survived and colonies that died. We quantified the proportional contribution of five major land cover types (deciduous forest, coniferous forest, grassland, cropland, and settlements) to the areas within a 2 km distance of the cavity trees based on a remotely sensed land cover map (Weigand et al., 2020) using GIS software (QGIS Development Team, 2021). The 2 km radii were chosen since approximately 80% of honeybee foraging takes place within this distance (Rutschmann, Kohl & Steffan-Dewenter, 2023) and because the landscape at the 2 km scale is known to have measurable effects on honeybee colony performance, including foraging rate, colony growth and winter survival (Steffan-Dewenter & Kuhn, 2003; Sponsler & Johnson, 2015; Rutschmann et al., 2022). A prior study on the spatial foraging behaviour of honeybee colonies in forest-dominated landscapes in Germany showed that the five land cover types differ in their relative value as foraging habitat, and therefore, differences in their contribution should correlate with differences in landscape-scale forage availability (Rutschmann, Kohl & Steffan-Dewenter, 2023).

Statistical analyses

All statistics were performed in R (R Core Team, 2022) and data figures were created using ggplot2 (Wickham, 2016). We compared parasite burden between surviving and dying colonies

based on three measures: the number of detected parasite taxa per colony, the community compositions of parasites, and the colony-level abundances of each assayed parasite taxon. Parasite numbers were analysed using a generalized linear model with a generalized Poisson error distribution and "winter survival" as a fixed factor (using the function "glmmTMB", (Brooks et al., 2017)). Since we had detected regional differences in parasite numbers in the original parasite study (Kohl et al., 2022) and since the distribution of winter survivals and deaths was not distributed equally across regions, "region" was a potential confounding factor. Therefore, we included "region" as a second predictor in the model (model formula: "glmmTMB(*Number of parasites* ~ winter survival + region, family = genpois)"). We tested for deviations from model assumptions using the functions "simulateResiduals", "testResiduals" and "testCategorical" from the "DHARMa" package (Hartig, 2022) and found none. To test the hypothesis that dying colonies had more parasite taxa than surviving colonies, we used a one-sided z-test ("glht" function from the "multcomp" package, (Hothorn, Bretz & Westfall, 2008)). To test for differences in parasite communities, we performed a distance-based redundancy analysis (dissimilarity measure: Jaccard distance) with winter survival as the constraining factor (function "dbrda" from the "vegan" package, (Oksanen et al., 2022)). Again, we factored out "region" as a potential confounding factor using the "Condition" argument (model formula: "dbrda(*Data frame of parasite prevalence* ~ winter survival + Condition(region), distance = "Jaccard)"). A permutation test was used to test whether there were non-random differences in parasite communities (99999 permutations, "anova.cca" function from the "vegan" package). We then determined, for both dying and surviving colonies, the prevalence and the mean, minimum and maximum colony-level loads of each parasite taxon, and used permutation tests to check whether there were associations between the colony-level parasite loads of any of the 18 parasite taxa and overwintering outcome (function "indepence_test" from the "coin" package, (Hothorn et al., 2016)). We used one-sided tests since the hypothesis was that dying colonies had higher parasite loads than surviving colonies.

We analysed the camera trap recordings using descriptive statistics. Based on the position of animals in relation to the cavity entrance on images and based on their behaviour as seen in the associated ten-second videos, we distinguished between cavity tree "visitations" and honeybee nest "intrusions". Due to the time-restricted recording scheme, it was not always easy to judge whether consecutive captures were independent (different visits/different individuals). We therefore considered the number of camera captures (images) per species as a measure of the interaction rate with the honeybee nests. To generate an overview of relative interaction rates among different species, we considered all captures taken by the 15 camera traps regardless of whether the cameras recorded during the whole examination period. For the nine camera traps with full coverage, we created summaries of interactions as a function of time, with the number

of captures binned by calendar week. We also provide the time course of average daily temperatures as obtained from two weather stations representative for the two study regions (Agrarmeteorologie Baden-Württemberg for St. Johann, www.wetter-bw.de, and Agrarmeteorologie Bayern for Birkenmoor, www.wetter-by.de). To test whether colonies protected with wire mesh had a higher winter survival rate than colonies in cavities without protection, we used a one-sided Fisher's exact test (function "fisher.test").

Results

Parasites and colony winter survival

The number of parasite taxa detected per colony was not higher in the colonies that died (mean: 4.9, range: 1–7, N = 57) than in the colonies that survived (mean: 5.7, range: 4–7, N = 10) (one-sided z-test: z = 1.537, *P*= 0.938, Figure 4.2a). The distance-based redundancy analysis revealed that parasite community compositions did not differ significantly between dying and surviving colonies (Permutation test: *P*= 0.352). This is illustrated by an ordination plot in which parasite communities of surviving colonies are completely nested within the ordination space of the parasite communities of dying colonies (Figure 4.2b).

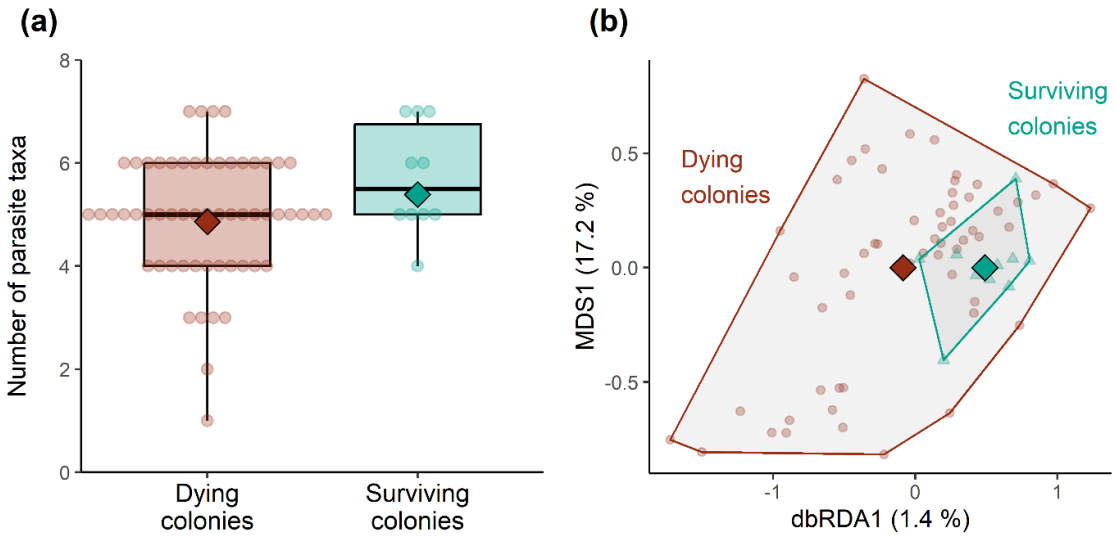

Figure 4.2: Comparison of parasite burden in dying (N = 57) and surviving (N = 10) feral honeybee colonies in the preceding summer. **(a)** Number of parasite taxa detected per colony among the 18 microparasites assayed. Diamonds on top of boxplots give model-estimated means, and dots are raw data. **(b)** Graphical representation of relative differences in parasite community composition as created by a distance-based redundancy analyses with "winter survival" as the constraining factor (effect of region partialled out). Percentages for the constrained axis (dbRDA1) and the first unconstrained axis (MDS1) give the share of explained community variation. Diamonds are means and dots (dying colonies) and triangles (surviving colonies) represent parasite communities of individual colonies.

Looking at each microparasite in detail, we detected 13 of the 18 microparasites assayed at varying prevalences (Table 4.1). Dying colonies did not have significantly higher loads than surviving colonies of any of the assayed parasite taxa (one-sided permutation tests, $P \geq 0.18$; see Table 4.1 for *P*-values of individual tests).

Table 4.1: Comparison of prevalence and colony-level parasite loads (Log_{10}[n / 100 ng RNA+1]) of 18 microparasites in D: dying colonies (N = 57) and S: surviving colonies (N = 10). Column "*P*" gives the *P*-values obtained from one-sided permutation tests of associations between parasite load and winter mortality.

			Parasite load			
Parasite Taxon	**Sample**	**Prevalence (%)**	**Mean**	**Min**	**Max**	***P***
Acarapis woodi	D	0	0	0	0	NA
	S	0	0	0	0	
Crithidia/ Lotmaria	D	77.2	5.6	0	8.8	0.959
	S	100	7.4	6.6	8.3	
Nosema apis	D	8.8	0.5	0	9.4	0.188
	S	0	0	0	0	
Nosema ceranae	D	96.5	6.8	0	9.6	0.308
	S	100	6.3	3.3	9.4	
Bacteria						
Melissococcus plutonius	D	5.3	0.1	0	2.5	0.858
	S	10	0.3	0	3.2	
Paenibacillus larvae	D	0	0	0	0	NA
	S	0	0	0	0	
Viruses						
Acute bee paralysis virus	D	54.4	3.2	0	8.2	0.941
	S	90	5	0	7.9	
Black queen cell virus	D	94.7	5.2	0	8.3	0.517
	S	100	5.3	4.4	6.1	
Chronic bee paralysis virus	D	1.8	0	0	2.7	0.338
	S	0	0	0	0	
Deformed wing virus A	D	1.8	0.1	0	4.3	0.338
	S	0	0	0	0	
Deformed wing virus B	D	19.3	1.2	0	8.6	0.833
	S	30	2	0	7.3	
Invertebrate iridescent virus 6	D	0	0	0	0	NA
	S	0	0	0	0	
Israeli acute paralysis virus	D	3.5	0.1	0	1.6	0.820
	S	10	0.1	0	1.5	
Kashmir bee virus	D	0	0	0	0	NA
	S	0	0	0	0	
Lake Sinai virus	D	86	4.8	0	7.3	0.764
	S	90	5.4	0	7.1	
Sacbrood virus	D	45.6	1.9	0	7	0.238
	S	30	1.3	0	6.5	
Slow bee paralysis virus	D	0	0	0	0	0.992
	S	10	0.2	0	1.8	
Varroa destructor macula-like virus	D	0	0	0	0	NA
	S	0	0	0	0	

Observations of cavity tree visitation and honeybee nest depredation

Camera traps installed at 15 trees captured 1263 usable images between September 2019 and the beginning of May 2020, with capture frequencies ranging between 0 and 10.8 captures per tree per week. They revealed that black woodpecker cavities occupied by feral honeybee colonies are regularly visited by a range of vertebrates involving at least 13 bird species and two mammal species during winter (Figure 4.3 and Figure S4.2).

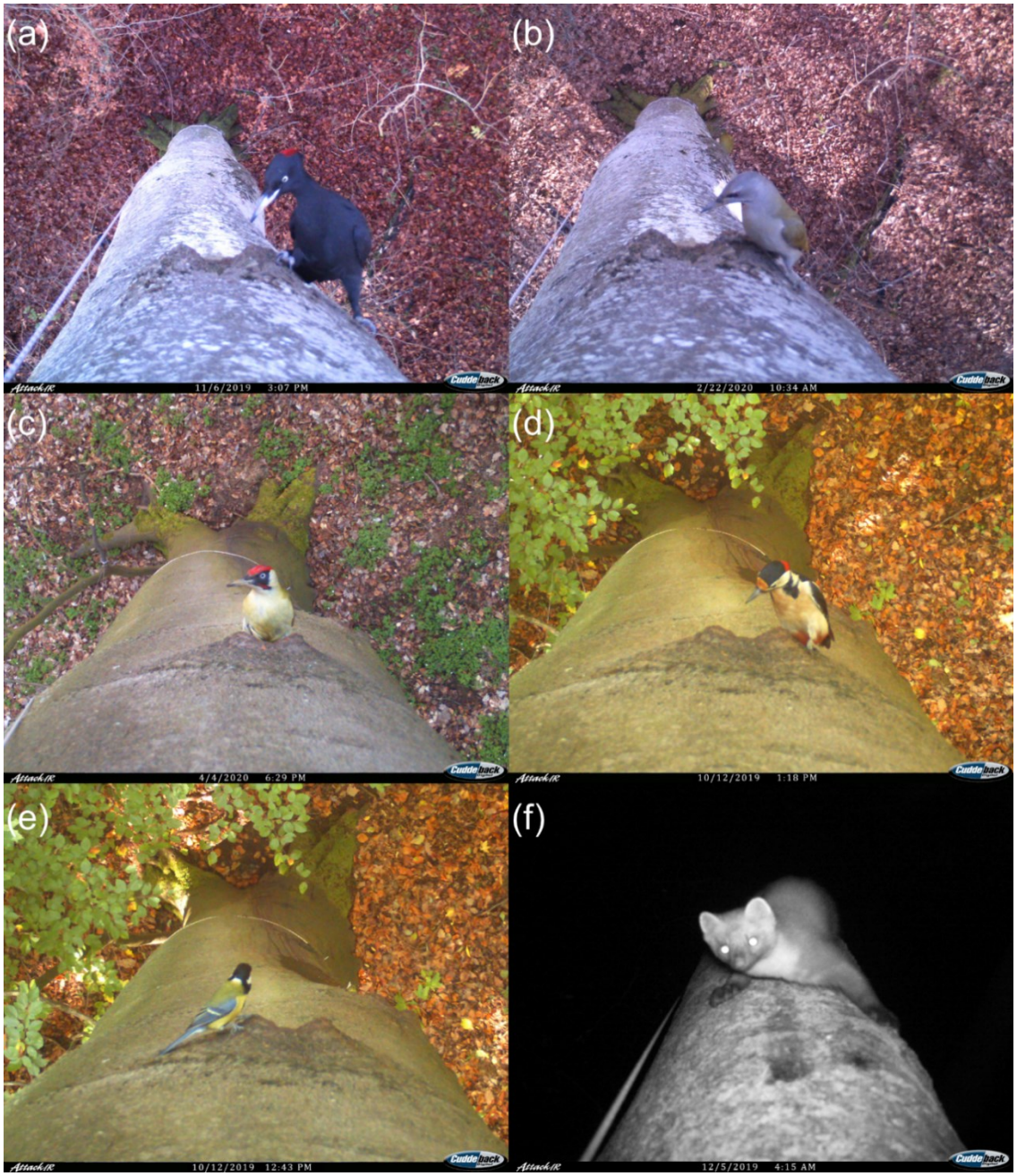

Figure 4.3: Camera trap images of six important winter visitors and depredators of honeybee nests in black woodpecker cavities. **(a)** Black woodpecker (*Drypocopus martius*), **(b)** grey-headed woodpecker (*Picus canus*), **(c)** green woodpecker (*Picus viridis*), **(d)** great spotted woodpecker (*Dendrocopus major*), **(e)** great tit (*Parus major*) and **(f)** pine marten (*Martes martes*). See supplementary information Figure S4.2 for images of the other visitors.

In 41% of the captures, visitors entered the bees' cavities with at least one body part and thus potentially plundered the nests. The featured taxa contributed to tree visitation at different proportions and differed in their propensity to intrude into the cavities (Figure 4.4).

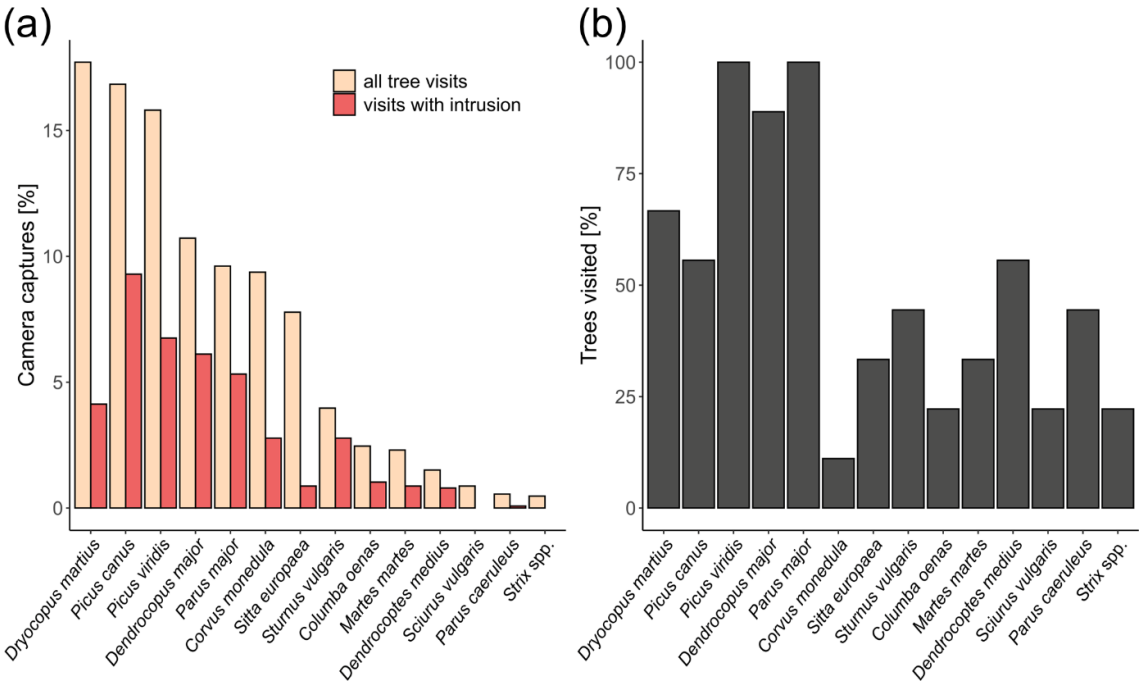

Figure 4.4: Relative contribution of 14 vertebrate taxa to bee tree visitation and honeybee nest intrusion during winter. **(a)** Proportion of camera captures per visitor taxon. "All tree visits" refers to all visits to the cavity trees captured by camera traps; "visits with intrusion" is a subset denoting cases in which animals entered the cavity of the bees with at least one body part. Data from all fifteen bee trees with camera traps. **(b)** Proportion of trees visited by each taxon. Data from nine bee trees continuously monitored with camera traps.

Plotting the average frequency of camera captures as a function of time revealed a bimodal activity distribution (Figure 4.5). The first peak of tree visitation in mid-November (greater than six captures per tree per week) likely mirrored an increased search for shelter in preparation for winter and/or the onset of targeted honeybee nest depredation. At the beginning of autumn, the frequency of cavity intrusion negatively correlated with temperature, indicating that potential depredators only entered cavities once the bees had ceased flight activity. The phase of relatively low tree visitation activity between mid-December and mid-February can be explained by cold temperatures and associated energy-saving behaviours of the animals. The second activity peak in March (>10 captures per tree per week) likely resulted from increased nest site search in preparation for the breeding season.

Based on the behaviour of the visitors as observed in 10-second videos (see supplementary videos at Dryad: doi:10.5061/dryad.jh9w0vtg7), the species-specific seasonal distribution of intrusion events (supplementary Figures S4.3 and S4.4), and natural history knowledge of the species' typical breeding sites, we distinguished between depredators and species that most likely only

visited cavities in search for shelter or nest sites without directly harming the bees. Six species likely preyed upon honeybees and their nests. Grey-headed woodpeckers (*Picus canus,* Figure 4.3b), green woodpeckers (*Picus viridis,* Figure 4.3c), great spotted woodpeckers (*Dendrocopus major,* Figure 3d) and middle spotted woodpeckers (*Dendrocoptes medius,* Figure S4.2e), although not usually using black woodpecker cavities for nesting (Sikora, Schnitt & Kinser, 2016), were observed sitting at the cavity entrances and pecking at combs throughout the observation period. Great tits (*Parus major,* Figure 4.3e) deliberately entered the cavities of every monitored bee tree and pecked at combs from October onwards. Lastly, pine martens (*Martes martes,* Figure 4.3f) were observed vigorously reaching into the honeybee nests with their forelegs or completely entering the cavities at four trees in November and February suggesting that they directly destroyed and potentially consumed honeybee nest content.

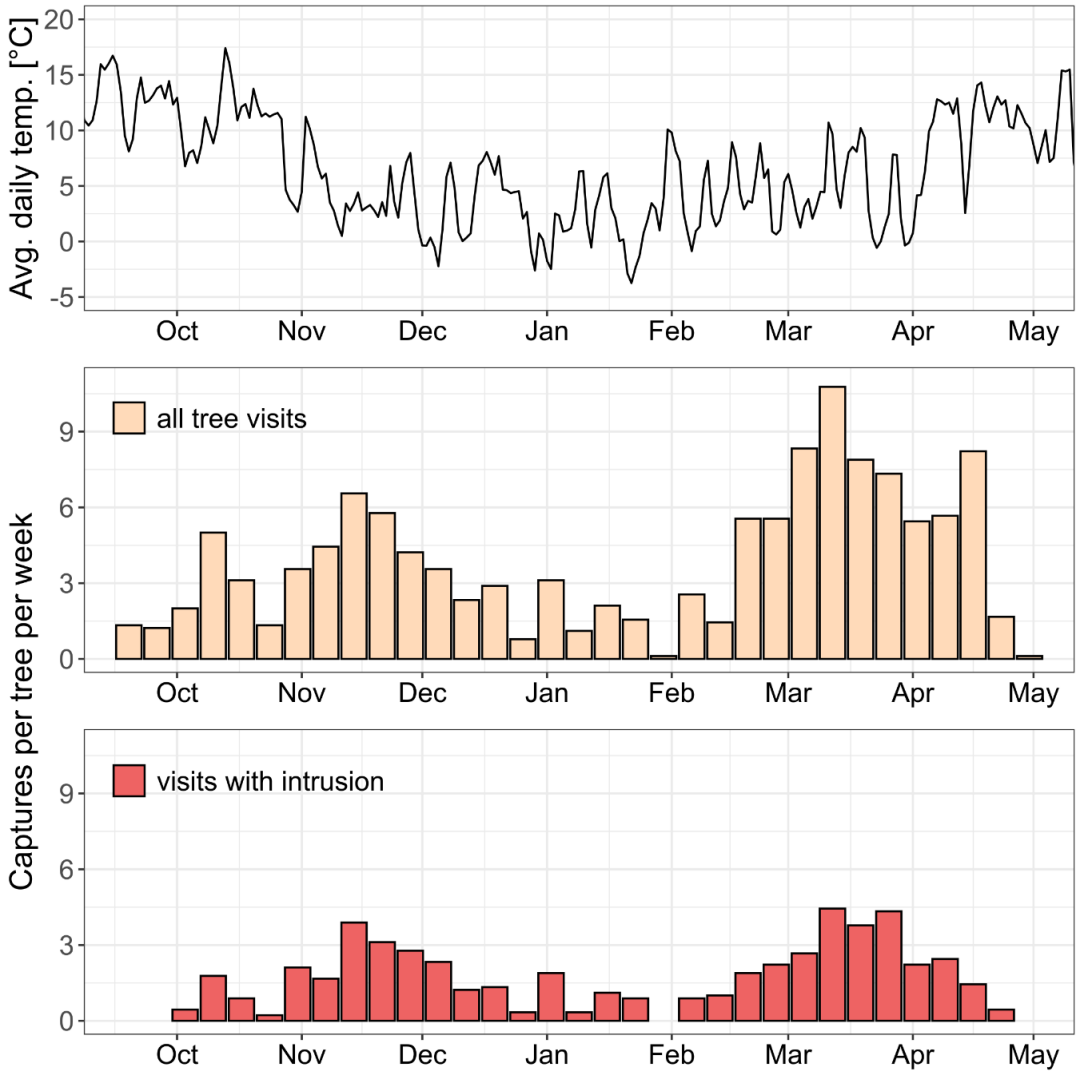

Figure 4.5: Time course of average daily temperatures and bee tree visitation frequencies by vertebrates between mid-September 2019 and early May 2020 as revealed by camera traps. Temperature data are averages obtained from two weather stations. Visitation frequencies are averaged over nine cavity trees for which we had full coverage (key as in Figure 4.4).

Eight other species could not be clearly classified as honeybee nest depredators. The most frequent visitor species, the black woodpecker (*Dryocopus martius,* Figure 4.3a), rarely entered the cavities before March. In comparison with the other woodpecker species, they showed a rather cautious exploration behaviour and rarely pecked at the bees' combs. Blue tits (*Parus caeruleus,* Figure S4.2g) displayed a similar behaviour as great tits but were only rarely observed. If they are nest depredators, they do not play an important role. The other visitors (Figure S4.2), namely jackdaws (*Corvus monedula*), nuthatches (*Sitta europaea*), starlings (*Sturnus vulgaris*), stock doves (*Columba oenas*), red squirrels (*Sciurus vulgaris*) and owls (*Strix* spec.), are all known to use black woodpecker cavities as resting or nesting sites. Since they were either infrequent visitors or only entered and cleared cavities in spring, they were most likely searching for nest sites rather than prey.

Effect of depredator exclusion on winter survival

Honeybee colonies in nests with mesh-protected entrances had a survival rate more than twice as high (33%) as control nests (15%) in winter 2019/20 (Figure 4.6a); however, the treatment and control groups had the same winter survival rate of only 10% in winter 2020/21 (Figure 4.6b). Taking the results of both years together (Figure 4.6c), the winter survival rate of colonies in protected nests (18.75%) was 0.5 times higher than that of unprotected colonies (12.5%), albeit this difference was not statistically significant (Fisher's exact test, P= 0.342).

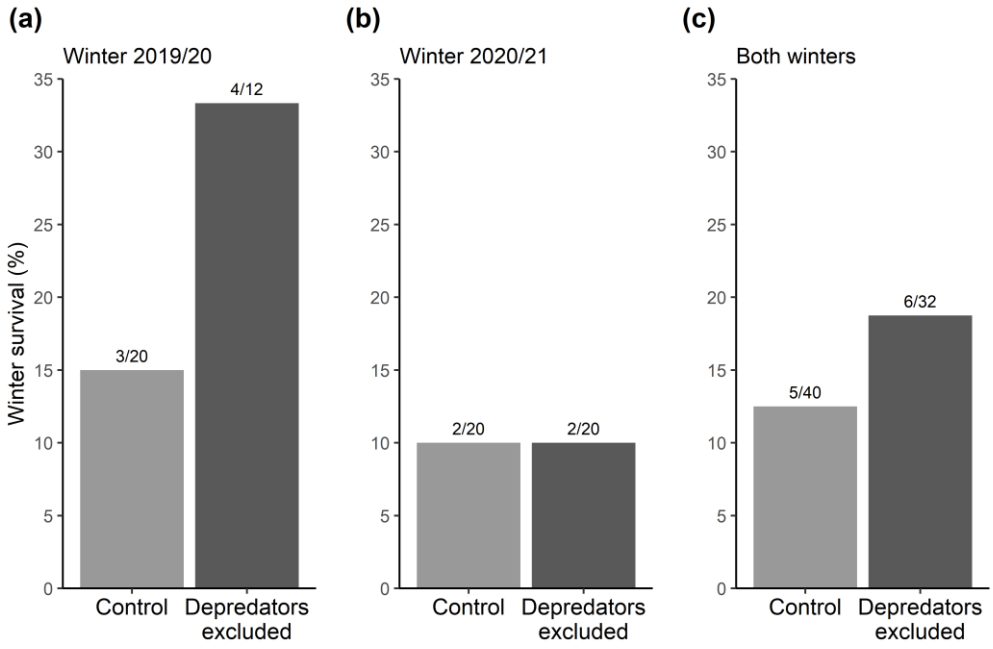

Figure 4.6: Results of the depredator exclusion experiments. Winter survival rates of colonies nesting in cavities with either open (control) or mesh-protected entrances (depredators excluded). Shown are the results for the experiments conducted in winter 2019/20 **(a)**, in winter 2020/21 **(b)**, and for both years pooled **(c)**. Numbers on top of the bars give the number of surviving colonies and the total number of cases.

Landscape context and winter survival

A redundancy analysis revealed that the composition of the surrounding landscapes – as described by the proportions of five major land cover types within 2 km radii – differed between the nest sites of dying and surviving colonies (Figure 4.7a). The difference was driven by the relative proportion of cropland and was not very likely due to chance ($P = 0.08$). Direct comparisons for each of the three study regions Swabian Alb, Coburg/Lichtenfels and Weilheim-Schongau showed that the average proportion of cropland was 4.9, 3.8 and 10.6 percentage points (mean: 6.4 points) higher in landscapes surrounding surviving colonies than in landscapes surrounding dying colonies (Figure 4.7b).

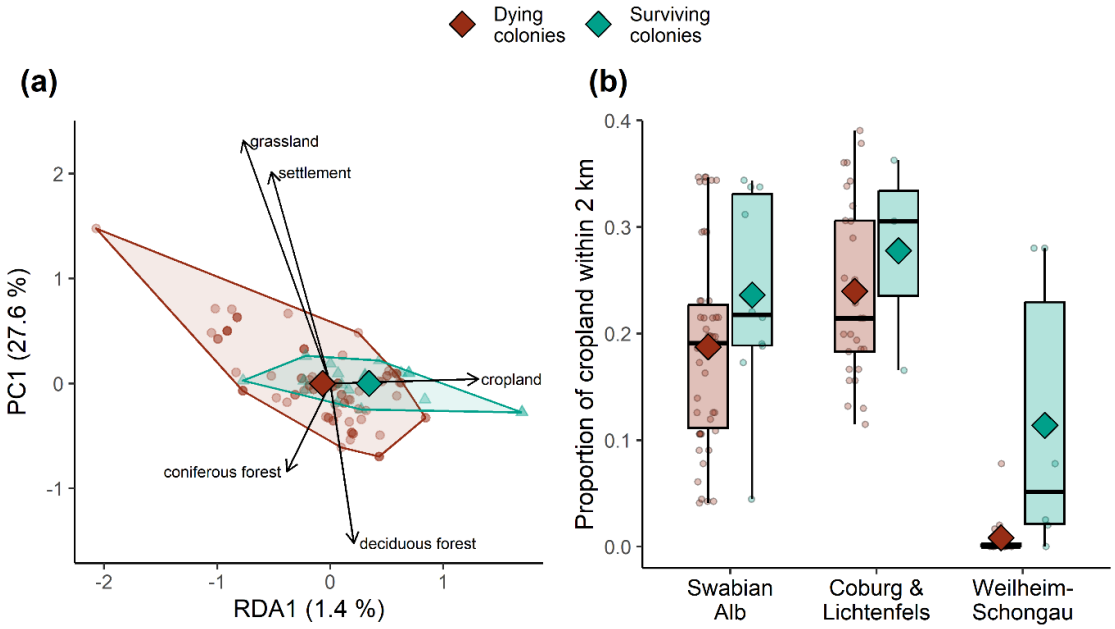

Figure 4.7: Comparison of circular landscapes (radius: 2km) surrounding dying (N = 94) and surviving (N = 19) feral honeybee colonies. **(a)** Differences in the composition of landscapes as revealed by a redundancy analysis with winter survival as the constraining factor (regional differences partialled out). Percentages give the share of landscape variation explained by the constrained axis (RDA1) and the first unconstrained axis (PC1). Diamonds are means, and dots (dying colonies) and triangles (surviving colonies) represent the landscapes surrounding individual colonies. The five arrows represent the correlations of the five land cover types with the ordination axes (RDA1 is correlated with the proportion of cropland). **(b)** Comparison of the proportion of cropland in landscapes surrounding dying and surviving colonies for each of the three study regions. Dots are raw data and diamonds are means.

Discussion

Feral honeybee colonies populating managed forests in southern Germany have extremely low chances to survive the winter. Investigating the causes of this high winter mortality can provide insights into the drivers of historical declines of wild honeybee populations and is needed for the design of effective conservation measures. Using feral colony overwintering observations gathered during a monitoring study, associated data on parasite prevalence, observations and experiments on nest depredation and landscape analyses, we made a first exploration of whether antagonistic interactions and/or landscape-level food limitations might explain winter mortality. A lack of difference in parasite burden between dying and surviving colonies suggests that winter mortality is currently not primarily caused by microparasites. Based on camera trap recordings, it seems more likely that avian and mammalian depredators are frequently involved in destroying feral honeybee colonies. Furthermore, the tendency of surviving colonies to be found in landscapes with relatively high proportions of flower-rich land cover suggests that forage availability determines winter survival chances. The hypothesis resulting from these observations is that the availability of protective nesting cavities and the provision of bee forage are currently more important than parasites in hampering feral honeybee population establishment in German forests.

Winter colony losses of managed honeybees are mainly explained by parasites (Genersch et al., 2010; Dainat et al., 2012b) and, therefore, the hypothesis that increased parasite pressure is also an important factor for feral colony overwintering is well justified (Thompson et al., 2014). However, apicultural management changes honeybee ecology in several ways that make managed colonies more likely to develop high parasite loads than wild-living colonies, e.g., by producing high local colony densities (Seeley & Smith, 2015; Nolan & Delaplane, 2017) and by keeping large colonies in unnaturally large hives (Loftus, Smith & Seeley, 2016). Furthermore, beekeepers support colony maintenance but prevent colony reproduction, thereby creating honeybee populations with a higher mean colony age. Since older colonies have higher parasite loads (Kohl et al., 2022) but most wild-living colonies die at an age of less than one year (Kohl, Rutschmann & Steffan-Dewenter, 2022), it needs to be scrutinized whether parasites represent a significant threat relative to other factors that kill colonies earlier in their lives. We considered here three measures of parasite burden of feral colonies and did not find any difference between colonies that died and colonies that survived the subsequent winter. The resulting conclusion is that parasites are currently not responsible for the high winter mortality. This would be incorrect if the parasite loads of feral colonies were above the thresholds typically leading to colony death, i.e., if all colonies were prone to die anyway. We think this scenario is very unlikely, given that, in our study system, the parasite burden of feral colonies was lower than that of sympatric managed

colonies (Kohl et al., 2022), and that the winter survival rate of managed colonies in Germany is generally much higher (80–96%; Genersch et al. 2010, Johannesen et al. 2022) than that of feral colonies (16%; Kohl et al. 2022a). A more serious caveat is that parasite burden in summer (we sampled colonies in July) might be a poor predictor of parasite-induced colony mortality in the subsequent winter. Quantifying parasite burden in late September or October, right before hibernation, would have been more informative (Dainat et al., 2012b). However, our analyses were not unreasonable because parasite burden in summer affects the health of winter bees which are produced from August onwards (Mattila, Harris & Otis, 2001), and a link between parasite burden in July and winter mortality has been demonstrated before (Ravoet et al., 2013). One could argue that there were important parasites which we did not test for. For example, we did not consider the infestation levels of the ectoparasitic mite *V. destructor*, which is commonly associated with managed colony losses (Genersch et al., 2010; Dainat et al., 2012b; Traynor et al., 2020). Quantifying mite abundances in feral colonies was not feasible due to limited access to bees and brood, but we think that this is not a serious limitation. On the one hand, mite infestation levels are known to highly correlate with the abundance of deformed wing viruses (DWV) (Dainat et al., 2012b; Norton et al., 2021), and we tested for two common strains of DWV. On the other hand, the damaging effect of *V. destructor* is mostly due to the transmission of DWV, not due to the direct damage by the mite (Di et al., 2016; Roberts et al., 2020), which is why DWV loads are more direct indicators of honeybee health than *V. destructor* infestation levels.

The lack of evidence of an association between parasites and winter survival in present-day feral honeybee colonies casts doubt on the widespread assumption that increased parasite pressure was historically responsible for the extinction of wild honeybee populations in Europe (Thompson et al., 2014; Meixner, Kryger & Costa, 2015). Our findings rather support the early statement of Stoeckhert that the lack of suitable nesting cavities was a major driver (Stoeckhert, 1933). Today, feral honeybee colonies regularly choose old cavities of the black woodpecker as nesting sites, but this does not mean that these homes are ideal for the bees. In fact, our camera trap recordings showed that black woodpecker cavities are frequented by a range of other cavity users during winter, implying that they are generally not safe places. The black woodpecker itself, stock doves, jackdaws, nuthatches, great tits, and pine martens have previously been shown to be common users of these cavities (Kosiński et al., 2010; Sikora, Schnitt & Kinser, 2016; Zahner, Bauer & Kaphegyi, 2017). In our study, most of these species were only seen entering the bees' cavities from March onwards, probably in preparation for the breeding season. At that time, late in winter, many honeybee colonies will have already died, so these visitors should not generally be classified as active nest-site competitors or depredators. The exceptions are great tits and pine martens. Great tits have long been known to be honeybee-eaters (Ambrose, 1997). While they usually occupy less than 10% of the available woodpecker cavities during the breeding season (Sikora,

Schnitt & Kinser, 2016), we observed that great tits entered every single monitored cavity from October onwards, suggesting that they actively searched for the bees and preyed upon them. Pine martens were observed on four trees only, but it has been recognised before that they are true depredators of honeybees (Hood & Caron, 1997). For example, Jedrzejewski et al. (1993) found that about 50% of pine marten scats contained remains of insects, with social wasps and bees (including *Apis*) making up the largest fraction, and Gunda (1968) reported that, historically, human bee-hunters in the Carpathian mountains used pine marten tracks in snow to locate bee trees, implying that the martens deliberately search for bee nests. Besides great tits and pine martens, we recorded four species which usually do not use black woodpecker cavities for nesting. Grey-headed woodpeckers, green woodpeckers, great spotted woodpeckers, and middle spotted woodpeckers clearly pecked onto the cavities and fed on the bee nest contents. While it is well known that green woodpeckers attack honeybee colonies (they also make holes in beekeeping hives) and that great spotted woodpeckers sometimes prey upon adult bees (Ambrose, 1997; Floris, Pusceddu & Satta, 2020), we now need to add the grey-headed woodpecker and the middle-spotted woodpecker to the list of honeybee enemies. These four woodpeckers plundered the cavities throughout the winter, suggesting that honeybees and their nests represent valuable caloric intakes for them. It was not possible to describe in detail the damage caused by the various intruders, but glimpses of the nests during the de-installation of camera traps revealed that, typically, parts of the combs were removed (supplementary information, Figure S4.5). However, it is still unclear whether the attacks were the actual causative factor for colony death. In the depredator exclusion experiments, honeybee colonies with protected entrances had a higher winter survival rate, but the difference to the control group with unprotected entrances was statistically not significant. However, these results are not yet conclusive. A technical flaw was that we only covered the entrances of the cavities and thus only prevented direct damage. This was unfortunate because the behaviour of the woodpeckers also involved hacking onto the outside walls of the cavities. It is known that physical disturbances of the nest can lead to colony arousal and increased winter food consumption which can be fatal when the food stores are small. Signs of recent woodpecker hacking around the entrances of mesh-protected cavities indicated that colonies that were supposed to be protected from enemies were probably also visited, and likely disturbed, by woodpeckers during winter (supplementary information, Figure S4.6). To properly test whether depredators affect overwintering, they need to be excluded not only from the cavity interior but from the whole tree section with the cavity. This could be achieved by wrapping the tree trunk with larger cages of wire netting. Another limitation of the experiment was the low number of replicates both in terms of mesh-treated cavities (N = 32) and study years (N = 2), and the fact that we considered the effect of nest depredation without controlling for other factors. For example, the low survival rate of only 10% among both the treatment and control groups in the

winter of 2020/21 suggests that other conditions necessary for winter survival were generally not fulfilled that year, potentially masking the effect of depredator exclusion.

A factor which likely affects feral colony winter survival regardless of depredation is the abundance of food in the surroundings during spring and summer (Rutschmann et al., 2022). Honeybee colonies need about 15 kilograms of stored food to survive the winter in Germany (personal observations). Hoarding enough honey is especially challenging for swarms that have founded a new nest because they require extra energy to build their beeswax combs and have little time to forage until the main nectar flows are over (Seeley, 2017). When considering that, among the feral honeybees colonising German forests, about 90% are recent founders (Kohl, Rutschmann & Steffan-Dewenter, 2022), and that finding pollen and nectar is especially challenging in German forests (Rutschmann, Kohl & Steffan-Dewenter, 2023) a critical role of food availability seems even more obvious. We were not able to directly analyse the effect of food abundance, but the positive association between winter survival and the relative proportion of a flower-rich land cover type is an indication that landscape-scale flower availability is an important driver of feral colony winter survival. When considering the same study region, colonies that survived the winter were surrounded by more cropland. Equating cropland and honeybee foraging habitat might seem overly coarse, given that this land cover type includes fields of any cultivated plant that is seasonally harvested. However, a functional relationship between the acreage of cropland and the landscape-scale availability of bee forage is indeed plausible. Both insect-pollinated crops like oilseed rape or sunflower and wind-pollinated crops like maize disproportionately contribute to the nectar and pollen intake of honeybee colonies (Requier et al., 2015). Furthermore, an analysis of the spatial foraging patterns of honeybee colonies based on the decoding of bee dances has shown that cropland is heavily overused by the bees (Rutschmann, Kohl & Steffan-Dewenter, 2023).

Studying wild-living honeybees in temperate forest landscapes is extremely challenging because low population densities (typically less than one colony per km^2) and nests high up in trees make it hard to find and access the bees (Kohl & Rutschmann, 2018; Seeley, 2019). We here used a unique set of colony observations to perform three independent tests of three contrasting drivers of feral colony winter mortality. Parasites, predators and food availability most likely have combined effects on colony survival (Dolezal et al., 2019) but, unfortunately, a simultaneous multi-factorial analysis was not feasible due to the limited number of colony observations and incomplete overlap in associated data. Another caveat was the naturally low ratio of survival versus mortality events, which took a toll on statistical power. Therefore, our study rather serves to redefine the likelihood of different hypotheses than to make final conclusions about the drivers of feral colony winter mortality. An important insight is that parasite burden is certainly less important than usually assumed. This is probably not because feral honeybees are not vulnerable

to parasites; rather, most colonies die at a young age when they do not (yet) suffer from high parasite pressure (Kohl et al., 2022; Kohl, Rutschmann & Steffan-Dewenter, 2022). The conservation implication is that certain habitat improvements can potentially foster wild-living honeybee populations regardless of parasites. Our camera traps revealed that at least five bird species and pine martens act as depredators of honeybee nests in black woodpecker cavities and this implies that the lack of optimal nest sites is a major problem. Subject to further investigations, any action that increases the abundance of large, well-protected cavities will probably improve the abundance and winter survival chances of wild-living honeybee colonies in managed forests. Next to the cavity-related problems, it remains highly likely after this study that food limitation explains parts of the feral colony winter losses. A promising way to further investigate the drivers of winter mortality is the use of artificial nest boxes. “Bait hives” with movable frames installed in trees have previously proven to be a valuable tool for the study of wild-living honeybees (Seeley, 2007, 2017). Excluding depredators, artificially feeding the colonies, controlling mite infestation, and taking samples of bees and brood for parasite screening are all straightforward with bait hives, allowing for full factorial study designs. However, controlled experiments should only complement, not replace, observations of honeybees nesting in natural cavities because otherwise we miss out on, and underestimate the effect of, the diverse ecological interactions that are excluded from man-made hives.

Acknowledgements

We thank Kurt Zeimentz for providing data on black woodpecker tree locations in Weilheim-Schongau and for encouraging us to perform the depredator exclusion experiment. We also thank Doris Rutschmann for lending us the electric staple gun used to fix protection grids over tree cavities. BR was supported by funds from the Bavarian State Ministry of Science and the Arts and the University of Würzburg to the Graduate School of Life Sciences (GSLS), University of Würzburg. We are grateful to Elena Reiriz Martínez for proofreading. This study was supported by a grant from the German Federal Environmental Foundation (Deutsche Bundesstiftung Umwelt, DBU) to PLK.

Supplementary information

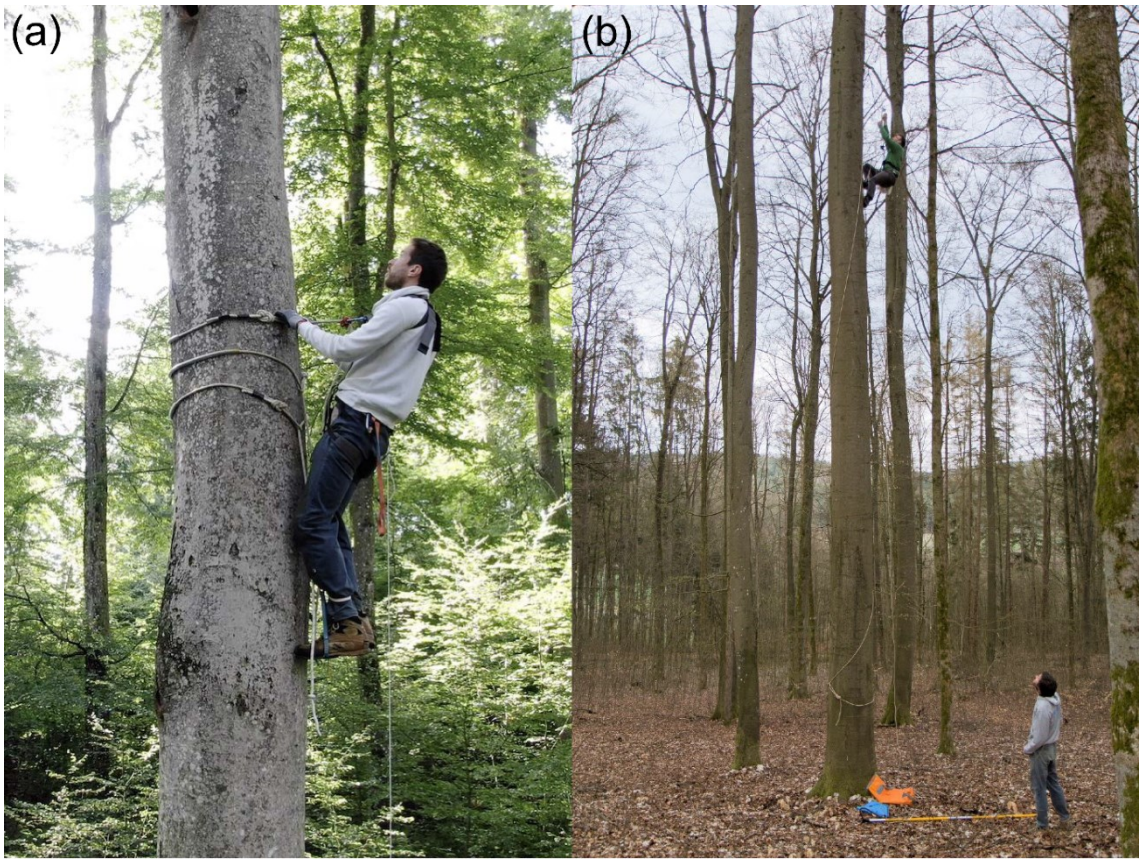

Figure S4.1: Methods of tree climbing used in this study. Trees were either ascended using a "trunk climbing" technique inspired by traditional tree beekeepers **(a)** or rope climbing **(b)**. For trunk climbing, three lassoes (one for the chest and one for each foot) were used to directly climb up tree trunks. For rope climbing, a semi-static climbing rope was pulled over a large branch high up in the tree and secured at the base of the tree trunk. The climbing person used a rope clamp to ascend the rope and a belay to descend. Figure (a) shows BR climbing up a tree on the Swabian Alb at the start of the experiment in 2020 (photo credit: Dimi Dumortier). Figure (b) shows PLK climbing up a tree in the county of Coburg at the end of the experiment in April 2021 (photo credit: Jean-Baptiste Pouchain).

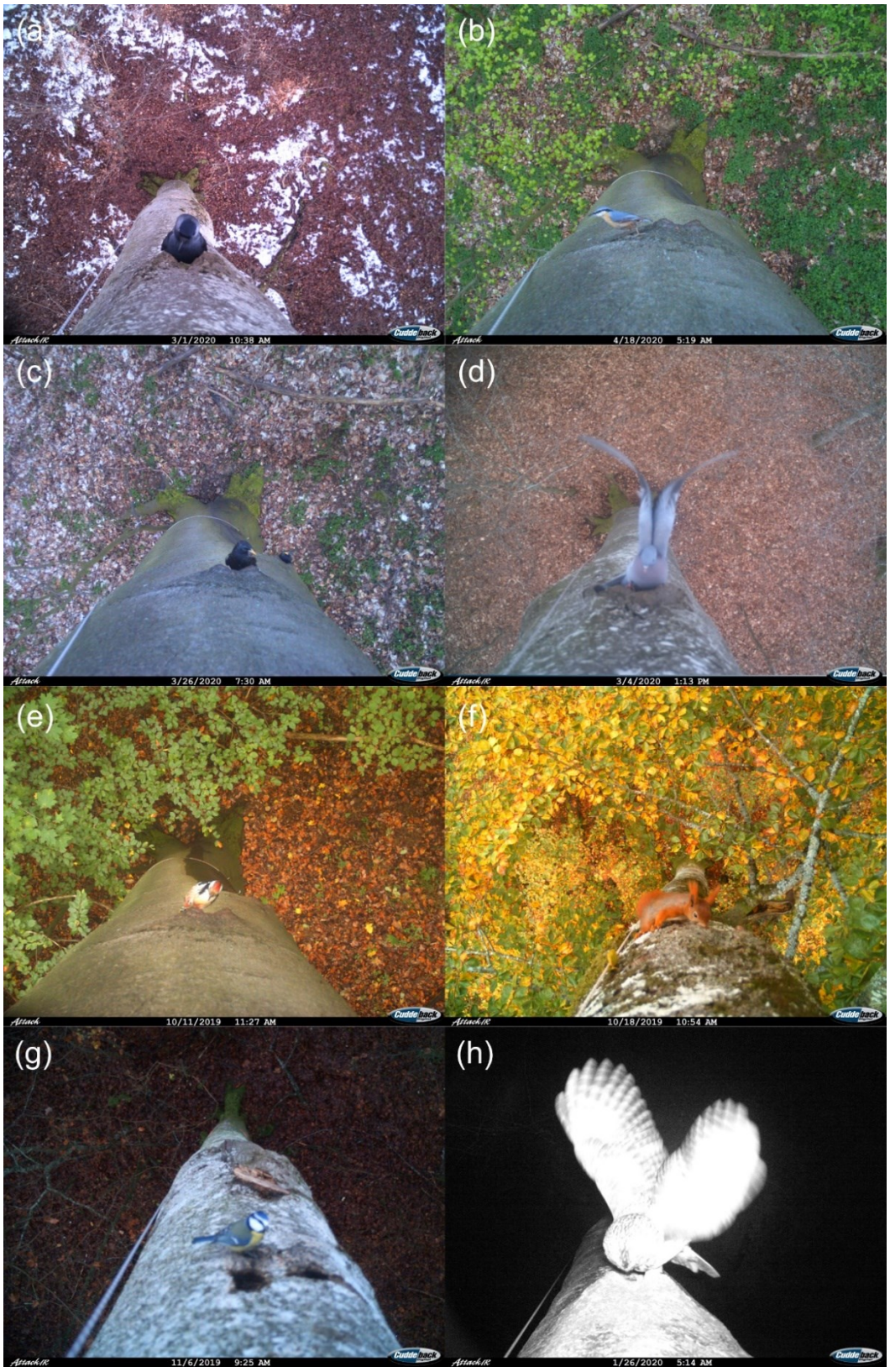

Figure S4.2: Camera trap images of another eight winter visitors of honeybee nests in black woodpecker cavities (see also Figure 4.3 in main text). **(a)** Jackdaw (*Corvus monedula*), **(b)** Eurasian nuthatch (*Sitta europaea*), **(c)** starling (*Sturnus vulgaris*), **(d)** stock dove (*Columba oenas*), **(e)** middle spotted woodpecker (*Dendrocoptes medius*), **(f)** red squirrel (*Sciurus vulgaris*), **(g)** blue tit (*Parus caeruleus*) and **(h)** an owl (*Strix* spec.).

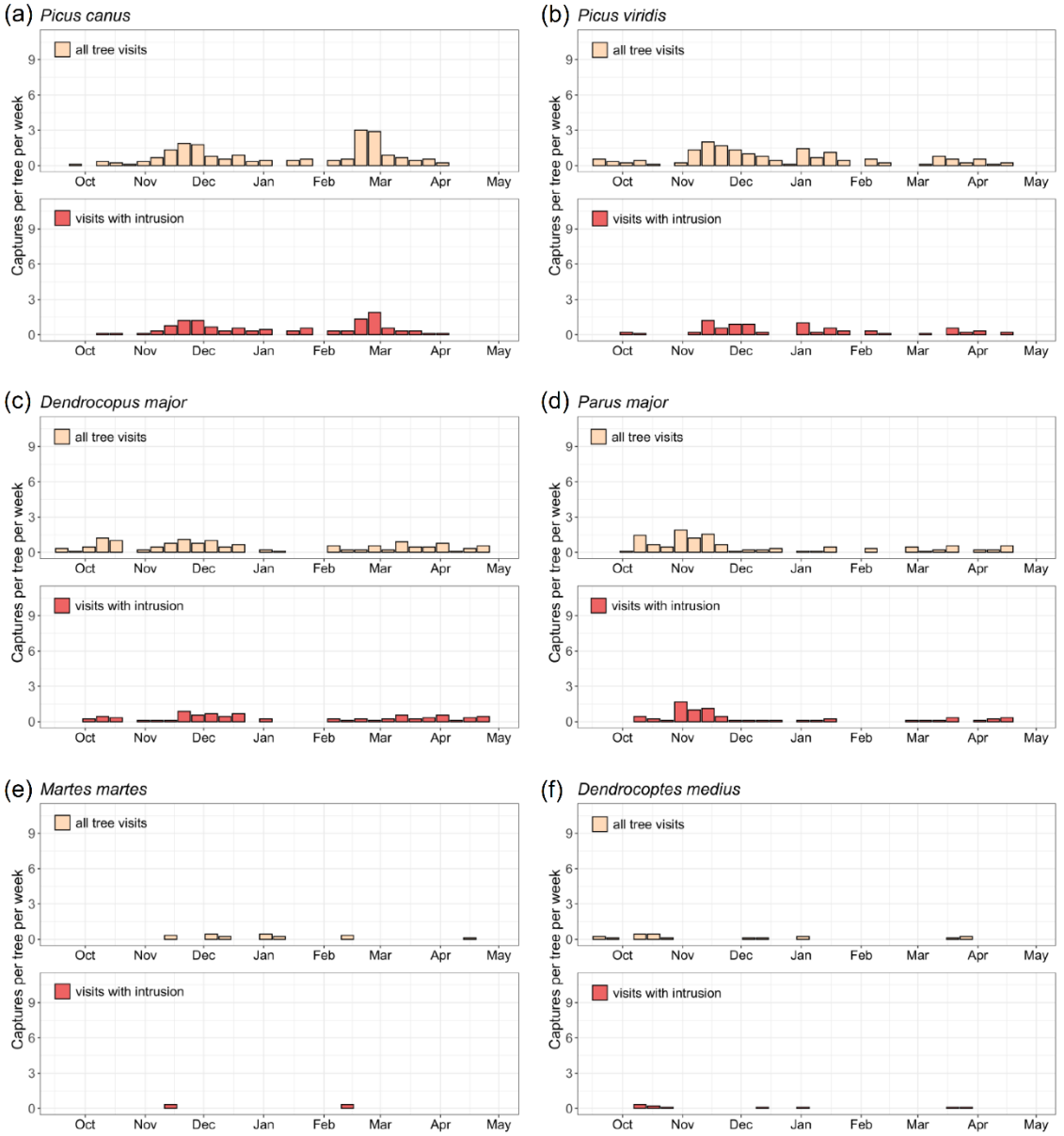

Figure S4.3: Time courses of bee tree visitation frequencies by different depredator species between mid-September 2019 and early May 2020 as revealed by camera traps. Visitation frequencies are averaged over nine cavity trees for which we had full coverage. The upper panels, "all tree visits", refer to all visits of the respective species to the cavity trees and the lower panels, "visits with intrusion", show a subset in which animals entered the cavity of the bees with at least one body part. Note that the behaviour of depredators is characterised by honeybee nest intrusion starting early in autumn. **(a)** Grey-headed woodpecker (*Picus canus*), **(b)** green woodpecker (*Picus viridis*), **(c)** great spotted woodpecker (*Dendrocopus major*), **(d)** great tit (*Parus major*), **(e)** pine marten (*Martes martes*), and **(f)** middle spotted woodpecker (*Dendrocoptes medius*).

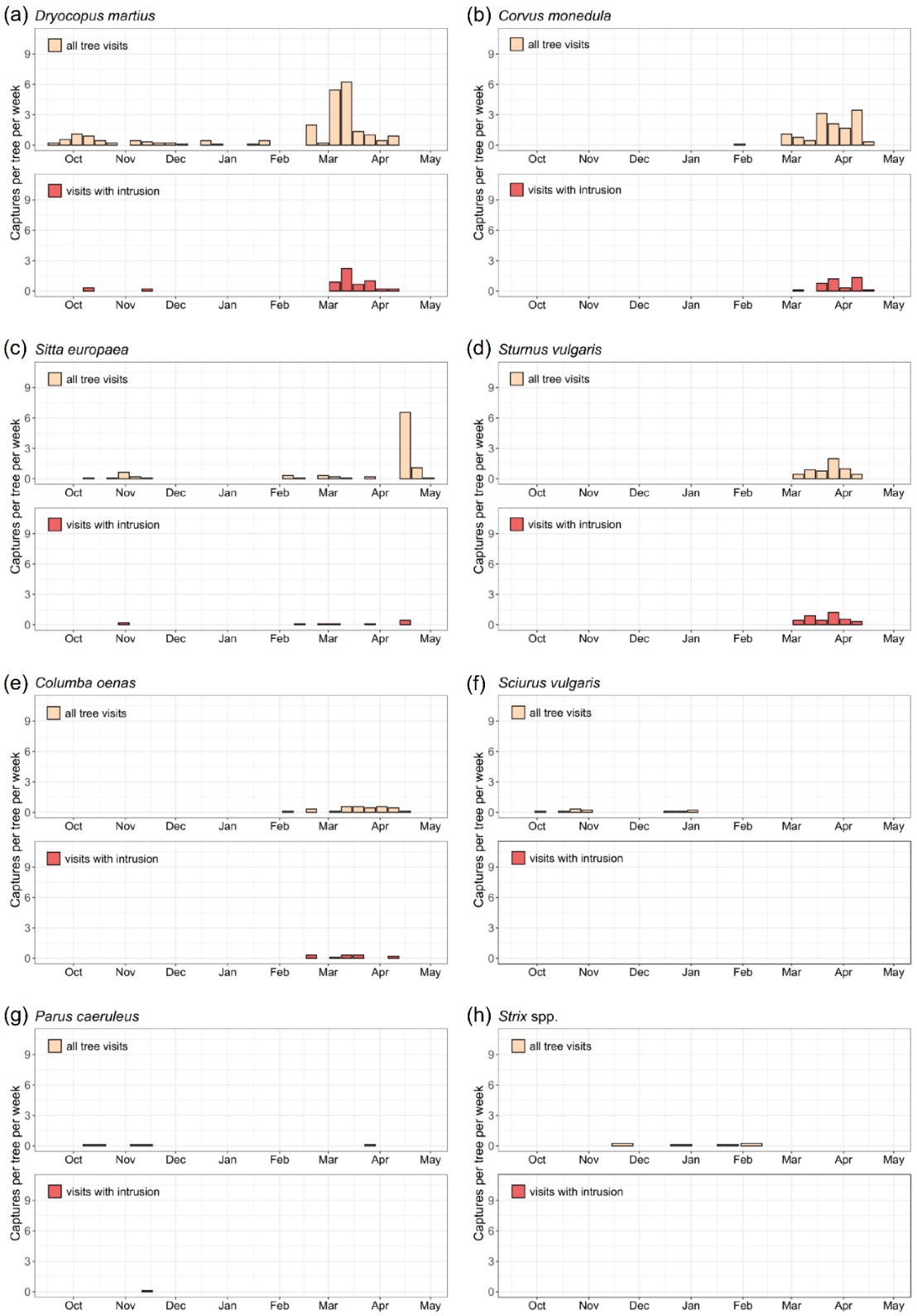

Figure S4.4: Time courses of bee tree visitation frequencies by different visitor species that were not classified as depredators (see Figure S4.2 for an explanation of the key). Note that cavity intrusions mostly happened in March, probably in preparation for breeding. **(a)** Black woodpecker (*Dryocpous martius*), **(b)** jackdaw (*Corvus monedula*), **(c)** Eurasian nuthatch (*Sitta europaea*), **(d)** starling (*Sturnus vulgaris*), **(e)** stock dove (*Columba oenas*), **(f)** red squirrel (*Sciurus vulgaris*), **(g)** blue tit (*Parus caeruleus*) and **(h)** owls (*Strix* spec.).

Figure S4.5: Image taken in April 2020 of the interior of a black woodpecker cavity that had been occupied by a honeybee colony. The bees died in winter and the cavity was taken over by a breeding pair of great tits (*Parus major*, note the clutch of eggs). The damages of the beeswax combs are typical. Photo credit: Benjamin Rutschmann.

Figure S4.6: The author (PLK) removing protection grids from a black woodpecker cavity in April 2021 (the cavity contained several entrances which needed to be sealed). Note the signs of woodpecker hacking on the margin of, and around, the main cavity entrance (the upper hole on the right side). These marks show that the treatment failed to prevent woodpeckers from working at the cavities during winter. Photo credit: Jean-Baptiste Pouchain.

Chapter five
General discussion

Honeybees in the forest, again and again

Based on the seminal finding that wild-living honeybees of European descent form stable populations in temperate forests in North America despite having been invaded by *Varroa* mites (Seeley, 2007, 2017), there is a growing interest in exploring whether wild populations might also persist in their native range. Within the last decade, the first systematic searches have revealed that wild-living colonies can indeed be found in various European countries (Oleksa, Gawroński & Tofilski, 2013; Kohl & Rutschmann, 2018; Requier et al., 2020; Browne et al., 2021; Dubaić et al., 2021; Moro et al., 2021; Oberreiter et al., 2021; Rutschmann et al., 2022). Sometimes, these studies have been interpreted as proof that the honeybee *still* exists in the wild (Seeley, 2019; Arndt & Tautz, 2020). By carefully monitoring pools of listed woodpecker cavity trees in German forests over up to four years, however, we have found that the survival rate of wild-living colonies (10.6%) is much too low for their populations to be viable (chapter one). Even when factoring in that surviving colonies will produce about two offspring (swarms) per colony in spring (net reproductive rate, R_0 = 0.318), wild-living populations would drop by about 99% in only four years. Evidently, at least today and at least for our study system in southern Germany, the occurrence of wild-living colonies is explained by the recurrent dispersal of swarms from colonies managed in apiaries. The wild-living honeybees can be confidently referred to as "feral" in the behavioural sense (Daniels & Bekoff, 1989); they are colonising tree cavities in forests *again and again*.

The insight that the studied cohorts of wild-living colonies are not self-sustaining means that they are not expected to differ biologically from the regional cohorts of managed colonies. In contrast to the bees of the Arnot forest in North America, which have evolved some resistance towards *V. destructor* (Mikheyev et al., 2016; Peck, 2018), we would not expect to find any defence mechanism in German feral colonies; they are as vulnerable as managed colonies.

One might wonder whether it is a novel phenomenon that wild-living honeybee colonies reliably occupy tree cavities in managed forests in Germany. Certainly, the structure of forests and the availability of nesting sites has changed tremendously throughout history (Küster, 1998). The abundance of cavity trees suitable for bees was probably at its lowest point between the mid-18th and late 19th centuries. On the one hand, existing old trees with natural or man-made cavities disappeared when multi-purpose silvopastures, which had been used for livestock grazing, tree beekeeping and as a source of wood, were transformed into dense, homogenous forests for the sole use of timber production. On the other hand, the black woodpecker as a creator of large cavities was very rare, even in high forests in Germany's low mountain ranges, because it was severely hunted for food and for its reputation as a forest pest (Schmidt et al., 2016). While the range expansion of the black woodpecker in Europe is still ongoing today(Gainzarain & Fernández-García, 2013), it probably reached its current densities in Germany only by about 1980 (Gerlach et al., 2019). Direct references are rare, but a photo of a honeybee colony living in a woodpecker cavity in a pine tree published by Zander (Zander, 1944, reprinted by Mittl, 2019) and the mention of colony observations in several forests of Franconia by Stoeckhert (1954) suggest that wild-living honeybees had (re-)colonised German forests by about 1950. At that time, the overall honeybee population had already been disturbed through introgressive hybridisation with non-native subspecies (Ruttner, 1992). However, the last major anthropogenic impact, the introduction of *V. destructor*, was still to come. Unfortunately, since there are no historical data, the question of whether wild-living honeybees nesting in black woodpecker cavities had higher survival rates before the invasion of the novel parasite will remain unresolved.

Comparative population demography of wild-living honeybees across Europe

It remains to be investigated whether the results obtained for the honeybees colonising managed German forests are representative of the current situation elsewhere in Europe. The population statuses of wild-living colonies might well vary between regions due to differences in environmental conditions and to biological factors relating to the bees themselves. One key insight of our work that is relevant for any other European study system, however, is that the rate at which swarms escape from managed hives is certainly much higher than previously thought of. We estimated that feral colonies make up about 5% of all honeybee colonies in our study regions in summer. Knowing that most of the feral colonies (about 70%) directly stem from managed hives and assuming that the escape of swarms is a common process, we can predict how many swarms will disperse from apiaries on larger scales each year. In Germany, which has approximately 1.1 million hives (Deutscher Imkerbund, 2020), about 40,000 swarms will become ownerless each spring, and in the whole of Europe, which has at least 18 million managed colonies (Phiri, Fèvre & Hidano, 2022), the annual number of honeybee escapees will be greater than

650,000. These figures, though only rough estimates, illustrate that the null hypothesis explaining the existence of any cohort of wild-living colonies must be the emigration of swarms from the regional cohort of managed colonies.

Due to the honeybee's long dispersal ranges, even colonies found in seemingly remote areas should not be accepted as evidence for a population independent of apiculture. Although honeybee swarms prefer to move to nearby cavities when they have the choice (Seeley & Morse, 1977), observations of the communication dances of nest-site scouts have shown that they search at distances of up to 10 km from their natal site (Camazine et al., 1999). Unfortunately, due to the great number of beekeepers and the high density of roads in Europe (Ibisch et al., 2016; Phiri, Fèvre & Hidano, 2022), managed hives can be installed virtually anywhere. There is hardly any spot that is out of reach for dispersing swarms.

A closer examination of other reports on wild-living honeybee colonies in Europe reveals that swarm dispersal from apiaries is typically the most parsimonious explanation. Oleksa, Gawroński and Tofilski (2013) discovered that wild-living colonies nest in old trees lining traditional avenues in northeastern Poland. The estimated colony density (0.1 colonies per km^2) is in the lower range of the values reported here and, since their rural study region probably contains many apiaries, managed hives are likely the source of these colonies. The systematic mapping of wild-living colonies in a German forest reserve, the Hainich National Park, by means of beelining also revealed a relatively low density of 0.13 colonies per km^2 (albeit this estimate is the minimum density) (Kohl & Rutschmann, 2018). Swarms experimentally installed around the Hainich found suitable nesting sites within a 500 m radius. However, the average distance between the wild-living colonies found in the forest and the apiaries in the surrounding villages, 2.7 km, is still well within the honeybee dispersal range. It also needs to be considered that some feral colonies *do* survive their first winter and certainly reproduce in the following spring. Therefore, even wild-living colonies nesting at sites twice or more the typical dispersal distance from managed colonies are no proof of a self-sustaining population.

An interesting study system was discovered by Dubaić et al. (2021). Based on citizen requests for the removal of honeybees in the metropolitan city of Belgrade, Serbia, they registered a total of 460 urban wild-living colony occurrences between 2011 and 2017. Since beekeeping is not yet very common in Belgrade, so the authors argue, the wild-living population is unlikely to depend upon the influx of swarms from apiaries. However, their map of Belgrade depicting the locations of the registered wild nests also shows the locations of more than 40 apiaries. In fact, the bee yards are distributed in such a way that no spot in the city is outside the dispersal range. Even assuming that swarms are unlikely to cross the Danube River, the most "remote" colony locations, in the historical centre of the city, are no more than 4 km from the nearest (registered) apiary. As

evidenced by the pioneering swarm observations of Lindauer in the city of Munich in Germany, dispersal distances of up to 4.5 km must be regarded as normal for urban areas (Lindauer, 1955). A follow-up study claiming genetic differences between wild-living and managed honeybees (Patenković et al., 2022) was not conclusive either, due to a spatial sampling bias. While the detection of genetic differences between cohorts of wild-living and managed honeybees would, in principle, be strong evidence for a self-sustaining wild population (Seeley et al., 2015) (note: the absence of a difference, conversely, would not be a negative indicator), it is crucial to base such a comparison on representative samples. Unfortunately, the sample of 40 wild-living colonies, neatly distributed over the city, was contrasted with a sample of 42 managed colonies stemming from only seven apiaries (Patenković et al., 2022). Just two of these apiaries laid within the area covered by the feral colonies. In such a constellation, group differences are likely biased by spatial patterns (higher genetic similarity of samples that are closer in space). Therefore, it should be scrutinised whether the historical centre of Belgrade simply acts as a sink of feral swarms due to the abundant nesting opportunities in old building walls.

In fact, a high density of wild-living colonies in urban areas is nothing uncommon. Besides various potential honeybee homes in man-made structures, older parks and green belts usually contain many more mature trees with cavities than forests managed for timber production (Figure 5.1). The long list of honeybee nest sites in trees and buildings generated by S. Roth and F. Remter for the city of Munich, which we capitalised on for the study presented in chapter three, is a good example. In the past few years, I also detected several nest sites in the “Ringpark” and adjacent green spaces in the city of Würzburg, a 50-hectare green belt marking the former city fortification. In the summer of 2021, I knew of five active nests, which translates into at least 10 colonies per km^2 – a density almost 50 times as high as our estimate for managed forests. However, we know that the survival rates of urban feral colonies in Germany are similarly low to those of the forest-dwelling colonies (own observations and personal communication by S. Roth).

Although these considerations show that a critical view is essential, there are at least indications that wild-living colonies are doing better in other regions. Thompson (2012) engaged the public to obtain information on the nest sites of wild-living colonies in England and followed 36 colonies over more than two years, from spring 2009 until autumn 2011. For this sample, the annual colony survival rate was 70–80%, which would be indicative of a stable wild population (with a swarming rate of two offspring per year, the threshold survival rate is at 33.33%). Browne et al. (2020) took a similar approach and followed the fate of 76 wild nests registered across Ireland from autumn 2015 until spring 2019. The reported survival times suggest an average colony lifespan of two to three years which, again, would clearly exceed the threshold required for a viable population (minimum average lifespan: one year). Do these studies show that the British Isles harbour self-sustaining populations of wild honeybees? Unfortunately, the monitoring

schemes were not sufficiently rigorous to reach this conclusion. The authors' selection of colonies was probably biased towards a larger share of established ones, which could have led to an overestimation of the overall survival rates. As the demographic studies of wild populations in North America and Australia showed (Seeley, 1978, 2017; Oldroyd et al., 1997), established colonies (older than one year) can have higher survival chances for the following year than newly founded colonies. Thompson (2012) specifically focused on established colonies, and the initial colony selection of Browne et al. (2020) likely had the same bias because citizens are more likely to spot nest sites with a history of continuous occupation. Furthermore, the time points of the censuses are only indicated vaguely: in both studies, nest sites were checked twice a year, in "spring" and in "autumn". And yet, the timing of particularly the spring census is crucial. On the one hand, colonies that are actively foraging in March and early April can still starve before the onset of the first nectar flow, so nest sites should not be checked too early. On the other hand, vacant cavities can quickly be re-occupied once swarming begins, so winter survival should not be determined too late. Finally, an unnoticed colony turnover can happen at any time during the swarming season between May and June, but the monitoring procedures were not robust against such false positive spring-summer survivals because colony continuity was not verified using genetic markers. Despite these limitations, which almost certainly led to the overestimation of demographic parameters, the high values reported still suggest that colony survival and lifespan are higher than in Germany.

Convincing evidence for regional differences in wild-living colony survival rates was delivered by Lang, Albouy, and Zewen (2022), who compared monitoring data from the metropolitan area of Dortmund in Germany (again, wild-living colonies in a city), the country of Luxembourg, and the County of Saintonge, a former province on the west Atlantic coast of France. In each study region, about 30 nest sites, equally distributed over cavities in buildings and trees, were monitored from 2018 until 2021 by making three to four visits per year. The annual colony survival rate for Dortmund was estimated to be 13.6% (N = 44 observations), the estimate for France was 34.6% (N = 104 observations), and the estimate for Luxembourg was somewhere between the former two but ambiguous due to missing data. The number for Dortmund is very close to our estimate of 10.6% for forest-dwelling feral colonies, indicating that the population is not self-sustaining What is remarkable is that the French population might well be at replacement level. Even though survival was probably slightly overestimated because, again, colony continuity over spring was not tested using genetic markers, the strong regional difference can be said to be proven.

Another case indicating that the situation in Germany is not necessarily representative of other regions is a cohort of wild-living colonies nesting in hollow electric poles in the province of Ourense in Galicia, Spain (Rutschmann et al., 2022). The first censuses conducted between autumn 2019 and spring 2021 suggest that winter survival is about 40% (N = 52 observations),

two to three times higher than the winter survival of feral colonies in Germany (16%, see chapter two). These comparisons show that it is worth continuing and increasing the efforts to collect demographic data on wild-living honeybees across Europe. In some regions, they might be forming cryptic, self-sustaining populations without our knowledge.

Figure 5.1: Bee tree (European ash, *Fraxinus excelsior*) in the Ringpark of the city of Würzburg. The entrance of the honeybee nest is marked with a red arrow. Veteran trees with irregular growth and cavities are abundant in old parks.

The potential impacts of feral honeybees on managed honeybees and ecosystems

Although Germany's wild-living honeybee populations are apparently not self-sustaining, they are probably not without effect on their environment. I will subdivide the potential ecological impacts of feral colonies into intraspecific effects and effects on other organisms.

The ecology of wild-living honeybees has traditionally received little attention but, when it has, the foremost question has been whether wild-living colonies harbour relatively more or relatively fewer parasites than managed colonies (Bailey, 1958). The argument for a higher parasite burden in wild-living colonies is that these are not monitored for disease by beekeepers and, since the invasion of *V. destructor*, not treated with miticides (Thompson et al., 2014). The hypothesised mechanisms explaining a lower parasite burden are reduced horizontal transmission due to well-dispersed nesting sites and reduced parasite population growth within colonies due to frequent swarming (which creates brood pauses and smaller colonies) (reviewed by Seeley, 2019). While experimental and theoretical studies had already shown that these factors are effective in reducing mite infestation levels (Seeley & Smith, 2015; Loftus, Smith & Seeley, 2016; DeGrandi-Hoffman, Ahumada & Graham, 2017; Nolan & Delaplane, 2017; Dynes et al., 2019), our direct comparison of feral and managed colonies suggests that they also help to reduce the prevalence of microparasites that live inside the bees' bodies. The feral colonies harboured significantly fewer microparasites among the 18 taxa tested than managed colonies (chapter three; Kohl et al., 2022). Unfortunately, our observational study does not allow us to completely separate the effects of nest spacing and swarming. Evidence for the effect of nest spacing stems from the comparison between newly (swarm-)founded feral colonies and swarms hived at apiaries, as these colony types only differ with respect to this single factor. Hived swarms managed at apiaries had a significantly higher parasite burden – they probably got reinfected by parasites stemming from adjacent managed colonies soon after their installation. However, pairwise comparisons between other colony types are less conclusive due to multiple ecological differences. For example, overwintered feral colonies and overwintered managed colonies differ not only in nest spacing but also in swarming (overwintered feral colonies probably issued swarms in spring) and in the time since their last mite treatment (greater than one year in feral colonies). Here, nest spacing and/or swarming at least compensated for the lack of mite treatment: there was no significant difference in parasite burden between these two colony types.

We can conclude from our data that wild-living colonies generally have a lower, certainly not higher, parasite burden than managed colonies. Therefore, they are unlikely to act as reservoirs or vectors of parasites and will not have a profound effect on disease prevalence in managed colonies. Undoubtedly, it sometimes happens that *V. destructor* is transmitted from a dying feral

colony to a managed colony through robbing behaviour (Peck & Seeley, 2019). However, managed colonies are much more likely to receive *Varroa* from other managed colonies (Frey & Rosenkranz, 2014; Seeley & Smith, 2015). Furthermore, feral colonies are unlikely to transmit parasites that are new to the cohort of managed colonies: we found no single parasite taxa that had a significantly higher prevalence among feral colonies than among managed colonies.

It is also worth mentioning that the bacterium *Peanibacillus larvae*, which causes the notifiable disease American foulbrood (Genersch, 2010), was not detected in any of the 64 feral colonies examined. This is interesting since it would have been conceivable that the long-lived spores produced by *P. larvae* accumulate in the tree cavities that are colonised year after year. The reason why this is apparently not the case might be that old combs in vacant nests are completely cleared by other cavity users and wax moths (Bailey, 1958; Ratnieks & Nowakowski, 1989), and that swarms thoroughly clean the nest interior and apply antimicrobial propolis after moving in (Seeley & Morse, 1976).

Our results are in line with studies conducted in Australia, New Zealand, and the USA, where similar or lower prevalence and/or infection levels of gut parasites, bacterial pathogens and/or viruses were found in wild-living colonies compared to managed ones (Goodwin, Houten & Perry, 1994; Manning et al., 2007; Youngsteadt et al., 2015). However, the outstanding value of our study is that we were aware of the population status of the wild-living colonies investigated: we knew that they formed non-sustaining, behaviourally feral cohorts. This is crucial, as it allows us to make statements about the sole effect of environmental and behavioural differences between managed colonies and wild-living colonies on parasite prevalence. In the other studies, which were conducted in regions where it is known (USA, Australia) or at least conceivable (New Zealand) that stable wild honeybee populations exist, the differences in parasite burden might also be caused by adaptive genetic differences between managed and wild colonies that evolved in response to the presence or absence of medical treatment by beekeepers (Mikheyev et al., 2016; Bozek et al., 2018; Peck, 2018). Our study clearly supports the premise that wild-living colonies are less vulnerable to parasites than managed colonies simply due to environmental differences. The beneficial effects on colony health of large distances between nests, and the small nests and lack of swarm control that lead to frequent swarming, apparently compensate for or even outweigh the negative effect of a lack of apicultural disease control. Despite much concern, feral honeybees appear to play a minor role in honeybee disease ecology.

Another potential intraspecific effect of feral colonies that has received little attention is their contribution to the regional pools of drones that young queens mate with. At first glance, there seems to be little reason to believe that the genetic contribution of feral colonies in Germany could be meaningful in any way. Only those feral colonies that have successfully overwintered would

be relevant in this context, but these only make up an estimated 0.5% of the regional honeybee populations in spring (chapter two). Another objection is that the drones of feral colonies should not qualitatively influence the honeybee gene pool since their mothers are anyway direct descendants of managed colonies. However, there are two arguments for why feral colonies could indeed matter. On the one hand, overwintered feral colonies will produce a disproportional abundance of drones, namely 5–6 times as many as typical managed colonies, because they nest in a natural comb nest in which about 20% of the comb contains cells for drone production (Allen, 1965; Kohl et al., 2015). In managed hives, drone production is suppressed through the use of worker cell wax foundation and/or the specific removal of drone brood. On the other hand, feral colonies that have managed to overwinter in the wild might not represent a random set of colonies drawn from the managed population. These are colonies that have escaped swarm control by beekeepers, successfully founded nests in suitable tree cavities, and accumulated enough honey to overwinter without receiving extra sugar water. Could it thus be that the annual feralisation of swarms and the survival and reproduction of a fraction thereof guarantees that genes adapted to life in the wild are maintained in the regional honeybee population? Many of the queens used by beekeepers in Germany stem from mating apiaries that are typically situated at sites remote from other bee yards, in forested and mountainous areas. At these sites, queens are supposed to mate with the drones of selected colonies that exhibit a combination of traits sought-after by beekeepers. We now know that feral colonies typically also live in these areas. It would be interesting to investigate which proportion of the offspring of queens that have mated at mating apiaries are sired by drones from local feral colonies. If feral colonies made a noteworthy contribution, I suppose the effect on the overall honeybee population would be rather positive, at least from a conservation perspective.

Considering the effects of feral honeybees on other organisms, one can distinguish between the pollination service provided by foraging bees (Hung et al., 2018) and the ecological interactions of natural honeybee nests in tree cavities (Saunders et al., 2021). The foragers of feral honeybee colonies certainly supplement the foragers of managed colonies spatially, with the latter being kept in the agro-urban space rather than in forests (Banaszak, 2009). My observations show that, when initiating a beeline from somewhere within a larger forest area, the chances are good to be guided to a feral colony, not to an apiary, which supports the assumption that forests would contain fewer foraging honeybees per unit area if feral colonies did not exist. However, in contrast to the production of drones, feral colonies do not contribute disproportionally to the density of foraging worker bees in the landscape. This means that feral honeybees mainly serve as pollinators from May and June onwards, when the density of colonies in forests has reached noteworthy numbers. Therefore, early-spring flowering species will not usually benefit much from feral honeybees, but forest species flowering in late spring, summer, and autumn, like *Rubus*

spp., *Frangula alnus*, *Tilia* spp., *Clematis vitalba, Epilobium angustifolium,* or *Hedera helix*, likely will. It would be interesting to know to what extent. This could be investigated by making flower observations and seed counts on plants at increasing distances from known honeybee nests (be they true feral colonies or experimentally established hives in the forest), or by comparing the seed set of open-pollinated flowers and flowers in which honeybees are specifically excluded (Wignall et al., 2020).

The foragers of feral colonies supplement those of managed colonies, but the colonisation of tree cavities by feral colonies represents a *qualitative* ecological supplement to apiculture because it leads to ecological interactions that are much less frequent or completely excluded from managed hives. Honeybee swarms generally compete with other cavity users for nest sites (Oldroyd, Lawler & Crozier, 1994; Paton, 1996; Pacífico et al., 2020; Saunders et al., 2021; Cunningham et al., 2022), and it has been observed that they can drive off stock doves from black woodpecker cavities (Reinsch, 1979). Once moved in, the application of antimicrobial propolis to the inner cavity walls may affect the further fungal decay of the cavity (Simone-Finstrom & Spivak, 2010). In autumn, after the nest is established and resources have been accumulated for winter, feral colonies become the target of various nest depredators including great tits, woodpeckers, and pine martens (chapter four). The organic detritus accumulating on the bottom of the nesting cavity and the leftovers of honey and pollen are exploited by a diverse community of arthropods and microorganisms associated with tree cavities (Figure 5.2). Finally, abandoned beeswax combs are the food of the caterpillars of two specialised lepidopterans, the lesser wax moth (*Achroia grisella*) and the greater wax moth (*Galleria mellonella*), both of which are pest species for beekeepers and usually excluded from comb material in apiculture (Williams, 1997). All these interactions are still (or again) occurring in German forests; for the commensals and depredators, it certainly makes no difference whether the bees form viable populations. However, almost nothing is known quantitatively about the effect of natural honeybee nests on other organisms in the forest. It would be interesting to explore how often birds like stock doves must abandon their nests because honeybee swarms choose to take their cavities. Birds and pine martens seem to feed on bees and combs, but it is unknown what exactly they consume, e.g., whether they also eat and digest beeswax, and how important this diet is during the winter months. The behaviour of such depredators might be revealed by camera trap observations in custom-made observation cavities in which artificial honeybee swarms are encouraged to build nests, and the contribution of honeybee nests could be estimated based on the analysis of bee remains in scats (Jedrzejewski, Zalewski & Jedrzejewska, 1993). Furthermore, the effect of honeybees on arthropods living in large tree cavities could be investigated by analysing their abundance and species composition in detritus samples from cavities with and without a history of honeybee occupation.

Figure 5.2: Ants (*Lasius fulginosus*) foraging on the leftovers of the honey stores of a honeybee colony that died in the winter. The photo was taken at a bee tree in early April 2020. For the ants, which are specialised in collecting honeydew, the finding must have been an early energy boost. Note the worker with the gaster full of honey in the centre of the image.

Which environmental factors hamper the establishment of wild honeybee populations in German forests?

Regarding the autecology of the wild-living honeybees, one of the most outstanding questions raised by this thesis is which environmental factors are responsible for their high colony mortality rate. Generally, three major external problems could be involved in limiting colony survival: lack of (high-quality) nesting cavities, lack of floral food resources, and parasite pressure. Problems related to the nesting cavities include the factor of nest depredation, since well-protected cavities should exclude enemies. (The process of animals preying upon individual bees outside the nest is not covered by this list. However, except for predation by the invasive oriental hornet, *Vespa velutina* [Requier et al., 2019], which has not yet colonised our study region, this factor is considered insignificant, at least in temperate climate regions [Morse & Flottum, 1997].) Different factors explaining colony mortality can interact, but there is a certain hierarchy among them. Parasite pressure probably only becomes relevant in high-quality habitats where colonies exist in a certain abundance. Consequently, the availability of nest sites and food resources are thought to be more decisive than parasite pressure in determining survival rate and population

size in wild honeybee populations (Bailey, 1958; Seeley, 1985; Ruttner, 1988a; Ratnieks & Nowakowski, 1989; Fries & Camazine, 2001; Seeley, 2019). In apiculture, however, where near-ideal nesting cavities and food are provided virtually ad libitum, parasite pressure is thought to be the most important limiting factor (Brosi et al., 2017; Traynor et al., 2020; Bartlett, 2022).

While it is a legitimate goal of beekeepers to achieve 100% colony survival in their apiaries each year, in a wild honeybee population, a certain rate of mortality is acceptable. Therefore, when trying to explain why the feral honeybee populations in German forests are not viable, it is important to define which portion of their colony mortality we should worry about. Demographic studies of two wild honeybee populations, from the Arnot Forest, USA (Seeley, 1978, 2017), and Wyperfield National Park, Australia (Oldroyd et al., 1997), showed that the average annual colony survival is only about 50%. The life-history characteristics of colonies from these disparate populations were remarkably similar, suggesting that they are typical for a stable population of wild honeybees. What needs to be explained, therefore, is the difference in survival between that 50% and the 11% observed among the German feral honeybees. Another important insight is that both in the Arnot Forest and in Wyperfield there was a clear difference in survival rate between newly founded colonies and established colonies (colonies older than one year) – a population characteristic we did not observe in Germany. Between both populations, the average annual survival rate of founders and of established colonies was 28% and 78%, respectively (Table 2.1 in chapter two). If we consider these figures as natural, the survival rate of feral colonies in Germany would need to be two to three times as high as it currently is in the case of founders and seven times as high as it currently is in the case of established colonies for the populations to become viable. Although most feral colonies die in their first year, the low survival rate of established colonies is especially concerning. In the following analyses, I will therefore also specifically consider whether any of the limiting factors can explain the absence of age-dependent survival differences in the German feral population.

From a beekeeping perspective, the most obvious factor explaining the high mortality among feral colonies would be a supposedly high parasite pressure due to the lack of treatment against *V. destructor* (Kraus & Page, 1995; Rosenkranz, Aumeier & Ziegelmann, 2010; Thompson et al., 2014; Traynor et al., 2020). However, as outlined above, the assumption that feral colonies have a high parasite burden is wrong, and we did not see a positive association between parasite pressure and feral colony winter mortality. We now know that most feral colonies die at an age of less than one year, when they typically have a very low parasite burden. Nevertheless, we might underestimate the role of parasites in killing established colonies due to their underrepresentation in the feral population. In fact, parasite pressure could be responsible for the lack of a difference in survival as a function of feral colony age in Germany. The reason why established colonies have a higher survival rate than founders in a “natural” wild population like that of the Arnot

Forest is thought to be that the founding period is especially resource-demanding (Seeley, 2019). Swarms need to build beeswax combs from scratch and use the remainder of the season to accumulate enough food, mainly honey, for the winter. Once they survive the first winter, colonies start the season with a substantial portion of their nest infrastructure already built and thus have more time and resources to store a surplus of honey for the subsequent hibernation phase. What is more, cavities occupied by established colonies have proven to be functional by having enabled successful overwintering at least once. Food availability and nest site problems are thus less likely to limit future survival in established colonies than in newly founded ones. The potential disadvantage of established colonies is that they have a higher parasite burden than newly founded ones, as we have shown in chapter three. Could it thus be that parasite pressure offsets the beneficial effect of accumulated resources on the survival rate of established colonies in Germany? An argument for this scenario is that the honeybees of the Arnot Forest and Wyperfield are indeed likely to be less vulnerable towards parasites. The Arnot Forest honeybees have been found to show increased brood hygiene and mite-grooming behaviour compared to unselected bees (Peck, 2018), so they possess some evolved defences against *V. destructor*. The bees in Wyperfield certainly face less parasite pressure than German feral bees because the population has not been invaded by the virus-vectoring mites (Traynor et al., 2020). It would be interesting to test experimentally whether the role of parasites as a limiting factor increases with colony age in wild-living colonies and, if so, at which age parasite pressure outweighs the role of resource limitation.

Besides the potential role of parasites later in the life of feral colonies, their effect on overall winter mortality is relatively minor, given that established colonies only make up a small fraction of the feral population. The lack of resources, in turn, is likely to explain a substantial portion of the winter mortality of all feral colonies (Parreño et al., 2022; Rutschmann et al., 2022). Food resource limitation is obvious when considering that the most common task of beekeepers when preparing managed colonies for overwintering is to ensure colony strength and food reserves, the latter of which is typically achieved by feeding them extra sucrose solution in late summer or early fall (Döke, Frazier & Grozinger, 2015). Differences in resource availability could also explain the overall differences in colony survival between Germany and the Arnot Forests or Wyperfield. The Australian *Eucalyptus* woodlands provide plenty of food for bees (Oldroyd et al., 1997), and the tree species richness of temperate forests in eastern North America is generally twice as high as in Europe (Latham & Ricklefs, 1993), so there are probably more species offering nectar and pollen in the Arnot Forest than in the forests of our study regions in Germany. In fact, observations of the tempo-spatial foraging patterns of honeybees show that beech-dominated forests in Germany represent an especially challenging habitat; colonies living inside the woods mainly forage in adjacent croplands and grasslands (Rutschmann, Kohl & Steffan-Dewenter,

2023). It was probably not a coincidence that feral colonies that had survived a winter were, on average, situated in landscapes with a higher proportion of flower resources-rich cropland.

At first sight, resource limitation alone seems unable to explain the lack of differences in annual survival between newly founded and established colonies. If food resources were the only factor, we would expect established colonies to be better off (Seeley, 2019). However, the advantage of established colonies regarding colony food reserves only applies to carbohydrates, i.e., beeswax and honey. Pollen, the protein source of the bees, is not stored in large quantities and needs to be continuously collected during the foraging season (Seeley, 1995). Interestingly, colony hive weight, which mainly reflects honey stores, is little affected by forest cover because forests do offer carbohydrates in the form of nectar and honeydew during short phases of the year. Pollen foraging distances, in turn, dramatically increase with increasing forest cover in the surroundings of colonies, especially in summer, indicating that feral colonies living in German forests might be especially protein-limited (Rutschmann, Kohl & Steffan-Dewenter, 2023). A shortage of pollen at any point in the season would affect newly founded and established colonies alike. It could hamper the production of winter bees and explain why colony survival rates are low regardless of colony age (Requier et al., 2017).

Release from parasites and abundant floral resources in the surroundings favour feral colony survival but are not sufficient if no suitable nesting cavities are available. Although the feral colonies investigated in the course of this thesis had successfully found and occupied a tree cavity, this does not mean that their nest sites were ideal. Cavities freshly excavated by the black woodpecker have a volume of about 10 L (Kosiński & Walczak, 2019), which is at the lower end of the range of acceptable cavity sizes. Seeley and Morse dissected 21 nests of wild-living colonies in trees and found that cavity volume ranged between 12 and 443 L, with most cavities holding 30 to 60 L (Seeley & Morse, 1976, 1978). Choice experiments in which artificial nest boxes of different sizes were offered to natural swarms showed that these prefer medium-sized cavities (40 L) over small (10 L) and large (100 L) ones (Seeley, 1977). This probably reflects a compromise between the need for honey storage space and the difficulty in regulating the nest temperature in very spacious cavities. Most of the woodpecker cavities monitored by us were not fresh but had already existed for several years, and many could be considerably enlarged through fungal decay. We also observed that the nest site selection of feral colonies was not random. It is therefore likely that the bees selected cavities with relatively larger volumes. Although we do not know the size of all monitored woodpecker cavities, preliminary measurements made by B. Rutschmann and me indeed indicate that those occupied by honeybees typically have greater volumes than the 10 L expected for freshly excavated ones.

Rather than offering too little nesting space, a problem of black woodpecker cavities could be that they have relatively large entrances: with a diameter of about 10 cm (Kosiński & Walczak, 2019), their entrance areas measure almost 80 cm^2. In choice tests offering nest boxes with either a small (12.5 cm^2) or large (75 cm^2) entrance area, honeybee swarms showed a clear preference for the former (Seeley & Morse, 1978). Small entrances facilitate the regulation of nest temperature, but what is certainly more important is that they offer increased protection from avian and mammalian depredators (Seeley, 2019). In fact, we saw in our camera trap observations that birds and pine martens had unlimited access to feral honeybee colonies, showing that black woodpecker cavities are far from being safe homes for the bees. Frequent nest depredation as an indirect cavity problem would also explain why newly founded and established colonies had similarly low winter survival rates. For the depredators, colony age certainly makes little difference. Furthermore, a critical role of nest depredation could explain the differences in colony survival rates among colonies in Germany, the northeastern USA, and Australia. In North America, there is no bird species known to actively prey upon honeybee nest content (Ambrose, 1997), and the sole mammal species that is a potential threat to honeybees nesting in tree cavities is the American black bear (*Ursus americanus*) (Hood & Caron, 1997). However, according to Seeley, who only observed a single black bear attack in many years of observing wild-living colonies in the Arnot Forest, black bears are not an important threat, probably because they have difficulties finding the bees' nesting cavities when these are high above the ground (Seeley, 2019). Among the native and introduced vertebrate fauna in Australia, there is, to my knowledge, no single species that would qualify as a honeybee nest depredator. Relatively large cavity entrances are therefore probably more problematic for wild-living colonies in their native than in their introduced range (Colautti et al., 2004). The nest site problem of limited protection against enemies is also likely to interact with the problems of resource limitation. Even if cavity intruders do not kill colonies in every circumstance, those that are frequently stressed might require more winter food and be more likely to starve. All in all, black woodpecker cavities seem at first sight an adequate refuge for wild-living honeybees in well-ordered managed forests, where trees rarely develop other types of cavities, but they might in fact represent ecological traps for the bees.

To summarise these considerations regarding the environmental factors limiting feral colony survival, we have established evidence of the role of nest site problems and food resource limitation. Black woodpecker cavities are probably not ideal nesting sites because they do not hinder the intrusion of nest depredators, and feral colonies will have difficulties finding enough forage, especially pollen, in managed forests in Germany (Rutschmann, Kohl & Steffan-Dewenter, 2023). An important role of parasite pressure is currently not supported by empirical data; however, a final judgement is not possible due to the suboptimal timing of parasite sampling, the lack of direct data on *V. destructor* infestation levels (see discussion in chapter four), and a

potential underestimation of the effect of parasites on established colonies. Certainly, multiple environmental conditions need to be fulfilled at the same time to enable feral colony winter survival. To understand the hierarchy among them and how they interact, we need to be able to better survey and manipulate feral colonies. Nest boxes mounted in trees that contain movable frames for colony inspection (Seeley, 2017) could serve as a tool for future studies aiming at investigating the ecological drivers of feral colony mortality.

Nature versus nurture: how much do intrinsic factors contribute to feral colony mortality?

It might be that the average environmental conditions in German forests are below the quality threshold required to sustain viable wild honeybee populations. However, factors intrinsic to the bees themselves might also contribute to their high mortality. In chapter one, I argued that colonies managed in beekeeping hives have basically remained wild organisms throughout apicultural history, because their reproduction has never been completely controlled. Seeing swarms become ownerless and observing honeybee foragers zooming in and out of tree cavities during these investigations has generally strengthened this view. However, it is also certain that the bees' gene pool has been undergoing some degree of anthropogenic modification for about 170 years, be it due to the introduction of non-native honeybee subspecies or to conscious and unconscious artificial selection (Rothenbuhler, 1958; Ruttner, 1992; Lodesani & Costa, 2003; De la Rúa et al., 2009; Requier et al., 2019a; Hoppe et al., 2020; Themudo et al., 2020). The critical question therefore is whether, or to which extent, the honeybees living in German apiaries have become genetically maladapted to life in the wild. Would the bees that originally lived in our forests until the widespread decline of tree beekeeping culture in the 18th century perform better under the current ecological circumstances?

Two mechanisms of human-mediated genetic change in the German honeybee population need to be considered separately: the introgression of genes from allochthonous bees, and artificial selection in situ (Rothenbuhler, 1958). From about 1850 onwards, colonies of non-native subspecies, mainly of the C evolutionary lineage, were massively introduced to Germany because these bees were more convenient to keep in the novel movable frame hives (Ruttner, 1992). By about 1950, beekeeping associations and apicultural scientists started to systematically replace the native European dark bee, *A. m. mellifera,* and its hybrids with Carnolian honeybees, *A. m. carnica* (Mittl, 2019). The result is that the extant honeybee population is mostly of Carnolian descent (Reinsch et al., 1991; Kauhausen-Keller & Keller, 1994; Francis et al., 2014). Could it be that colonies of *A. m. carnica* are naturally less well-adapted to life in the forests of central Europe north of the Alps than *A. m. mellifera*? When considering the general climate and the types of forest found in Germany and in the native range of *A. m. carnica*, there is no reason

to assume that these bees would have problems with the local environmental conditions. The *carnica* lineages propagated in Germany originally stem from regions in Austria and Slovenia with similar climate and forests that are also dominated by beech (*Fagus sylvatica*). It is the geographic mountain barriers rather than an environmental cline that maintained the natural separation of *A. m. mellifera* and *A. m. carnica* in central Europe (Ruttner, 1992). However, there are behavioural traits thought to differ between the two subspecies that might affect their survival chances in the wild. According Ruttner, Milner and Dews (1990), colonies of the European dark bee are characterised by a rather conservative life-history strategy. They are thought to start rearing brood relatively late in spring and maintain relatively moderate colony sizes throughout the season. Due to the storage of large quantities of pollen and honey close to the brood nest at any time, the maintenance of foraging activity at relatively low temperatures, and a quick reduction of the amount of brood as a response to periods of bad weather, *A. m. mellifera* appears especially well adapted to survive in regions with unpredictable weather and mediocre nectar flows (Ruttner, Milner & Dews, 1990; Büchler, 1998). I assume that these characteristics would be highly advantageous for colonies living in beech-dominated forests in Germany. In contrast, the rapid spring colony development typical of *A. m. carnica* colonies could be a handicap under feral conditions. In Germany, early periods of warm weather in March and April are often intermitted by cold snaps, during which colonies that maintain too much brood can easily fail to feed their larvae and starve without assistance from beekeepers (Dustmann & von der Ohe, 1988).

To test whether there are subspecies-specific differences in the likelihood of colony survival under feral conditions in German forests, experimental swarms headed by purebred *A. m. mellifera* or *A. m. carnica* queens would need to be installed in small hives in different forests, left unmanaged and monitored. Such experiments would need to involve queens of several sources per subspecies in order not to confound lineage effects with subspecies effects and (Büchler, 1998), ideally, be repeated over several years.

Apart from the potential effect of subspecies identity, artificial selection under modern beekeeping conditions might take a toll on the survival abilities of feral honeybees (Rothenbuhler, 1958; Lecocq, 2018; Hoppe et al., 2020). The classical traits consciously selected in breeding programmes are calmness, gentleness, low propensity to swarm and high honey yields (Hoppe et al., 2020). How calm bees sitting on the combs remain when drawn out of the hive is probably irrelevant to the performance of wild-living honeybee colonies. Increased gentleness would be a clear disadvantage if the depredator species were affected by bee stings. However, I suspect that a colony's defence strategy against nest depredation is the proactive selection of cavities with small entrances (Seeley & Morse, 1978) rather than stinging, because cold temperatures impede the flight of nest guards in winter. Selection against the propensity to swarm has not generally achieved to breed colonies that do not swarm at all. Therefore, swarming still needs to be

prevented by means of beekeeping manipulations: enlarging the nest space, removing swarm (queen) cells, and creating nucleus colonies. The trigger mechanism initiating colony reproduction in spring is the crowding of workers inside the nest (Smith, Koenig & Peters, 2017), and since feral colonies occupy cavities of limited volume, they probably swarm as frequently as they would naturally do. The selection for increased honey yield is interesting, as the amount of honey stored by honeybee colonies is a product of various environmental and behavioural factors. It is almost certainly not possible to increase the foraging efficiency of honeybee colonies through artificial selection because this is what they were naturally optimised for during millions of years of evolution (Moritz & Crewe, 2018). All environmental variables being equal (including resource availability and hive manipulations), the most significant colony trait positively affecting the amount of honey stored is colony size (Farrar, 1937; Harbo, 1986). Therefore, when selecting colonies that produce a lot of honey, beekeepers are unconsciously selecting for colonies that are largest at the times of the main nectar flows. Colonies best achieve this by initiating brood production very early in the season and showing explosive growth in spring. I therefore suppose that selection for honey yield has led to the exaggeration of what is thought to be a natural subspecies characteristic of *A. m. carnica*. The consequence of a shift in allocation from colony maintenance to quick colony growth would be that feral colonies, which cannot receive emergency feed from beekeepers, are more prone to starve in late winter or early spring.

Besides the traits directly selected for in bee breeding and the indirect selection of colonies that grow quickly, beekeeping with movable frame hives might have unconsciously selected for other characteristics that are maladaptive for colonies living in the wild. There is the general question of the extent to which managed honeybee populations are domesticated (Lecocq, 2018; Seeley, 2019). This issue is not only important for conservation programmes aiming at re-establishing wild honeybee populations. It is also highly relevant in the context of competition between honeybees and wild non-*Apis* bees and the conservation of plant-pollinator interactions. Investigating the evolutionary effects of modern rationalised beekeeping with movable frame hives requires the availability of pairs of sympatric (or near sympatric) honeybee populations in which one is managed and the other is proven to be wild (i.e., self-sustaining). With respect to temperate-adapted western honeybees, such pairs of populations can readily be found within the non-native range of the species (Oldroyd et al., 1997; Seeley, 2007), but true wild populations still need to be discovered in Europe.

The future of wild-living honeybees

When considering the current legal regulations regarding the western honeybee in Germany, one might think that it is no more than a livestock species. Veterinary orders oblige beekeepers to register their hives, monitor colonies for notifiable diseases and pests and treat them regularly

with miticides. The species conservation law lists all members of the superfamily Apoidea as strictly protected except for *A. mellifera*, which is explicitly considered to be domesticated (Mittl, 2017). The insight obtained from the presented investigations that tens of thousands of feral colonies occupy old woodpecker cavities and other hollow spaces each year shows that the biological reality of the honeybee is currently being neglected. Recognising the dual nature of the honeybee as both managed and wild living is equally important for apiculture and nature conservation (Requier et al., 2019a; Panziera et al., 2022).

It is considered an ethical responsibility of the owners of domesticated species to tend their animals, which includes providing them with shelter, food, and medical care, because human activity has made them vulnerable (Palmer, 2011). Therefore, from the normative perspective of the honeybee as a domesticated species, the consequence of our findings would be that we must find ways to completely prevent honeybee feralisation. To that end, we would either need to increase the frequency and intensity of beekeeping manipulations to control swarming or breed bees that do not show this behaviour anymore. I suppose that neither option is practically feasible, nor would they be accepted by the wider beekeeping community. The honeybees' natural behaviour of colony reproduction and dispersal by swarming, which happens sooner or later in any apiary, is the best proof that honeybee management is primarily based on culturally accumulated beekeeping techniques rather than the domestication of the bees (Seeley, 2019). Accepting this fact and adopting the perspective that the honeybee is both a managed and a wild species, however, does not preclude taking responsibility for feral colonies. On the contrary, it would set the ground for the systematic investigation of the factors that currently hamper wild honeybee population re-establishment.

By considering the honeybee through the lens of wildlife research, we will not only fulfil our responsibility towards feralised colonies but also gain valuable insights for nature conservation more generally. This is because, due to their multifaceted habitat requirements and their large home ranges, wild-living honeybees can be regarded as umbrella organisms, whose well-being is an indicator of general habitat quality (Rutschmann et al., 2022). For example, while parasite pressure has been cited as the main cause of wild honeybee population decline when considered through the lens of apiculture (Thompson et al., 2014; Meixner, Kryger & Costa, 2015), we now almost certainly know that the lack of suitable nesting cavities is a much more decisive factor (chapter four). Studying wild-living honeybees has revealed that our contemporary managed forests lack tree cavities that are large in volume but have small entrance holes. Such a microhabitat, offering both space and protection, is most likely found in veteran trees in which heart rot, knotholes or bark injuries have had time to develop into large hollows through the activities of fungi and saproxylic arthropods and is not only crucial for honeybees (Cockle, Martin & Wesołowski, 2011; Courbaud et al., 2022; Visick & Ratnieks, 2023). Unfortunately, large old

trees are extremely rare in human-dominated landscapes (Lindenmayer, Laurance & Franklin, 2012; Lindenmayer & Laurance, 2017).

It might turn out that the simple provision of nest boxes imitating large tree cavities could enable the establishment of viable wild-living honeybee populations, in the same way as small nest boxes are known to support the populations of hole-nesting birds and mammals (Newton, 1994; Lindenmayer et al., 2016; Cunningham et al., 2022; Figure 5.3). Such insight would free honeybees and apiculture alike because it would take the pressure off beekeepers to perform rigorous swarm controls. Instead, the installation of bait hives ready to be occupied by swarms could develop into a standard beekeeping praxis for (re-)obtaining colonies for the apiary (Seeley, Morse & Nowogrodzki, 1989; Villa et al., 2008). However, future investigations might also find that factors intrinsic to the bees are hampering their survival. In case our modern ways of beekeeping and bee breeding have led to the evolution of honeybees critically maladapted to life in the wild, we would need to question these practices. This is because, from a societal perspective, the function of beekeeping is to conserve the honeybee for its pollination services and not to domesticate it. We would also need to consider establishing large protection areas explicitly devoted to (the evolution of) wild honeybee populations (Requier et al., 2019a).

Figure 5.3: Artificial nest box made of woodstone that mimics a tree cavity. The well-insulated, small-entrance cavity is intended to exclude woodpeckers and to serve as a winter home for bats. The one in the image allowed a colony of feral honeybees to successfully overwinter (the colony was discovered in the summer of 2022 by the author and the photo was taken in March 2023). The close-up of the nest entrance in the top right corner shows two worker honeybees zooming in.

In the three rural study regions in which we conducted the monitoring of feral colonies, forests comprised less than 50% of the total land cover. Black woodpecker cavities were distributed over many fragments of forest interspersed by villages, grassland, and cropland, so feral colonies were usually within the dispersal and mating range of managed colonies. It is large continuous forests that are the least affected by the genetics of artificially selected managed honeybees. Despite novel pressures like *V. destructor*, large forests support wild honeybee populations in eastern North America (Seeley, 2019) and presumably also in the southern Ural, as the continued practice of tree beekeeping suggests (Ilyasov et al., 2015). Such evolutionary refuges of wild honeybee populations might still exist in central Europe today (Requier et al., 2020). The question is what forest size and quality can sustain an independent population of temperate-adapted honeybees.

In concluding these speculations on the future of wild-living honeybees, I hope that the research and thoughts presented in this thesis will prove valuable for others and that this line of research will not end here but rather unfold in many ways. Comparisons of our results with the findings of other studies from different parts of Europe suggest that populations of wild-living honeybees might be viable elsewhere (Browne et al., 2021; Lang, Albouy & Zewen, 2022; Rutschmann et al., 2022). The continued demise of tens of thousands of feral honeybee colonies occupying black woodpecker cavities should be a call to action, to try to understand under which circumstances wild honeybees could live in Germany.

References

Abd Al-Fattah MAAW, Yehia Ibrahim Y, Ibrahim Haggag M. 2021. Some biological aspects of honey bee colonies in relation to the age of beeswax combs. *Journal of Apicultural Research* 60:405–413.

Alaux C, Le Conte Y, Decourtye A. 2019. Pitting wild bees against managed honey bees in their native range, a losing strategy for the conservation of honey bee biodiversity. *Frontiers in Ecology and Evolution* 7:60.

Allen MD. 1965. The effect of a plentiful supply of drone comb on colonies of honeybees. *Journal of Apicultural Research* 4:109–119.

Ambrose JT. 1997. Birds. In: Morse RA, Flottum K eds. *Honey bee pests, predators and diseases*. Medina, Ohio: The A.I. Root Company, 341–356.

Arndt I, Tautz J. 2020. *Honigbienen - geheimnisvolle Waldbewohner*. München: Knesebeck.

Ayala AJ, Yabsley MJ, Hernandez SM. 2020. A review of pathogen transmission at the backyard chicken–wild bird interface. *Frontiers in Veterinary Science* 7:539925.

Bailey L. 1958. Wild honeybees and disease. *Bee World* 39:92–95.

Banaszak J. 2009. Pollinating insects as an example of changes in fauna. *Fragmenta Faunistica* 52:105–123.

Bartlett LJ. 2022. Frontiers in effective control of problem parasites in beekeeping. *International Journal for Parasitology: Parasites and Wildlife* 17:263–272.

Baudry E, Solignac M, Garnery L, Gries M, Cornuet J-M, Koeniger N. 1998. Relatedness among honeybees (*Apis mellifera*) of a drone congregation. *Proceedings of the Royal Society B* 265:2009–2014.

Berry JA, Delaplane KS. 2001. Effects of comb age on honey bee colony growth and brood survivorship. *Journal of Apicultural Research* 40:3–8.

Biesmeijer JC, Roberts SPM, Reemer M, Ohlemüller R, Edwards M, Schaffers a P, Peeters T, Potts SG, Kleukers R, Thomas CD, Settele J, Kunin WE. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* 313:351–354.

Blaker H. 2000. Confidence curves and improved exact confidence intervals for discrete distributions. *Canadian Journal of Statistics* 28:783–798.

Bolker B and RDCT. 2022.bbmle: Tools for general maximum likelihood estimation. (R package version 1.0.25.) *Available at https://cran.r-project.org/package=bbmle*

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

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. *bioRxiv*:498436.

Brittain C, Kremen C, Klein AM. 2013. Biodiversity buffers pollination from changes in

environmental conditions. *Global Change Biology* 19:540–547.

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 Journal* 9:378–400.

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:e02706.

Brosi BJ, Delaplane KS, Boots M, De Roode JC. 2017. Ecological and evolutionary approaches to managing honeybee disease. *Nature Ecology and Evolution* 1:1250–1262.

Browne KA, Hassett J, Geary M, Moore E, Soland-reckeweg G, Ferrari R, Loughlin E Mac, Brien EO, Driscoll SO, Young P, Pinto MA, Grace P, Browne KA, Hassett J, Geary M, Moore E, Soland-reckeweg G, Ferrari R, Loughlin E Mac, Brien O, Driscoll SO, Young P, Pinto MA, Mccormack GP. 2021. Investigation of free-living honey bee colonies in Ireland. *Journal of Apicultural Research* 60:229–240.

Büchler R. 1998. Vergleichende Untersuchung von Volksentwicklung, Flugaktivität und Pollensammelverhalten verschiedener *Apis mellifera carnica*- und *Apis mellifera mellifera*- Herkünfte. *Apidologie* 29:465–467.

Büchler R, Andonov S, Bienefeld K, Costa C, Hatjina F, Kezic N, Kryger P, Spivak M, Uzunov A, Wilde J. 2013. Standard methods for rearing and selection of *Apis mellifera* queens. *Journal of Apicultural Research* 52:1–30.

Büchler R, Costa C, Hatjina F, Andonov S, Meixner MD, Le Conte Y, Uzunov A, Berg S, Bienkowska M, Bouga M, Drazic M, Dyrba W, Kryger P, Beata P, Pechhacker H, Petrov P, Kezić N, Korpela S, Wilde J, Conte Y Le, Uzunov A, Berg S, Bienkowska M, Bouga M, Drazic M, Dyrba W, Kryger P, Panasiuk B, Pechhacker H, Petrov P, Kezić N, Korpela S, Wilde J. 2014. The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera* L. colonies in Europe. *Journal of Apicultural Research* 53:205–214.

Büchler R, Uzunov A, Kovačić M, Prešern J, Pietropaoli M, Hatjina F, Pavlov B, Charistos L, Formato G, Galarza E, Gerula D, Gregorc A, Malagnini V, Meixner M, Nedić N, Puškadija Z, Rivera-Gomis J, Rogelj Jenko M, Smodiš Škerl MI, Vallon J, Vojt D, Wilde J, Nanetti A. 2020. Summer brood interruption as integrated management strategy for effective *Varroa* control in Europe. *Journal of Apicultural Research* 59:764–773.

Budge GE, Barrett B, Jones B, Pietravalle S, Marris G, Chantawannakul P, Thwaites R, Hall J, Cuthbertson AGS, Brown MA. 2010. The occurrence of *Melissococcus plutonius* in healthy colonies of *Apis mellifera* and the efficacy of European foulbrood control measures. *Journal of Invertebrate Pathology* 105:164–170.

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. *Nature Communications* 11:2164.

Burnham PA, Alger SA, Case B, Boncristiani H, Hébert-Dufresne L, Brody AK. 2021. Flowers as dirty doorknobs: Deformed wing virus transmitted between *Apis mellifera* and *Bombus impatiens* through shared flowers. *Journal of Applied Ecology* 58:2065–2074.

Bütler R, Lachat T, Krumm F, Kraus D. 2020. Habitatbäume kennen, schützen und fördern. In: *Merkblatt für die Praxis 64*. Birmensdorf: Eidg. Forschungs anstalt WSL.

Calfee E, Agra MN, Palacio MA, Ramírez SR, Coop G. 2020. Selection and hybridization shaped the rapid spread of African honey bee ancestry in the Americas. *PLoS Genetics* 16:e1009038.

Camazine S, Visscher PK, Finley J, Vetter RS. 1999. House-hunting by honey bee swarms: Collective decisions and individual behaviors. *Insectes Sociaux* 46:348–360.

Carpenter MH, Harpur BA. 2021. Genetic past, present, and future of the honey bee (*Apis mellifera*) in the United States of America. *Apidologie* 52:63–79.

Carvell C, Bourke AFG, Dreier S, Freeman SN, Hulmes S, Jordan WC, Redhead JW, Sumner S, Wang J, Heard MS. 2017. Bumblebee family lineage survival is enhanced in high-quality landscapes. *Nature* 543:547–549.

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. *Parasitology Research* 114:651–658.

Chang SY, Hoopingarner RA. 1991. Relative importance of feral honey bees in apple pollination. *Acta Horticulturae* 288:239–243.

Chauzat MP, Cauquil L, Roy L, Franco S, Hendrikx P, Ribière-Chabert M. 2013. Demographics of the European apicultural industry. *PLoS ONE* 8:e79018.

Chen C, Liu Z, Pan Q, Chen X, Wang H, Guo H, Liu S, Lu H, Tian S, Li R, Shi W. 2016. Genomic analyses reveal demographic history and temperate adaptation of the newly discovered honey bee subspecies *Apis mellifera sinisxinyuan* n. ssp. *Molecular Biology and Evolution* 33:1337–1348.

Cockle KL, Martin K, Wesołowski T. 2011. Woodpeckers, decay, and the future of cavity-nesting vertebrate communities worldwide. *Frontiers in Ecology and the Environment* 9:377–382.

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ. 2004. Is invasion success explained by the enemy release hypothesis? *Ecology Letters* 7:721–733.

Conte Y Le, Meixner MD, Brandt A, Carreck NL, Costa C, Mondet F, Büchler R. 2020. Geographical distribution and selection of European honey bees resistant to *Varroa destructor*. *Insects* 11:873.

Costanzi L, Brambilla A, Di Blasio A, Dondo A, Goria M, Masoero L, Gennero MS, Bassano B. 2021. Beware of dogs! Domestic animals as a threat for wildlife conservation in Alpine protected areas. *European Journal of Wildlife Research* 67:70.

Council of Europe. 2004. COUNCIL REGULATION (EC) No 797/2004 of 26 April 2004 on measures improving general conditions for the production and marketing of apiculture products. *Official Journal of the European Union* 47:1–3.

Courbaud B, Larrieu L, Kozak D, Kraus D, Lachat T, Ladet S, Müller J, Paillet Y, Sagheb-Talebi K, Schuck A, Stillhard J, Svoboda M, Zudin S. 2022. Factors influencing the rate of formation of tree-related microhabitats and implications for biodiversity conservation and

forest management. *Journal of Applied Ecology* 59:492–503.

Covarrubias-Pazaran G, Diaz-Garcia L, Schlautman B, Salazar W, Zalapa J. 2016. Fragman: An R package for fragment analysis. *BMC Genetics* 17:62.

Crane E. 1990. *Bees and beekeeping: science, practice and world resources*. Heinemann Newnes.

Crane E. 1999. *The world history of beekeeping and honey hunting*. London: Duckworth.

Cunningham SA, Crane MJ, Evans MJ, Hingee KL, Lindenmayer DB. 2022. Density of invasive western honey bee (*Apis mellifera*) colonies in fragmented woodlands indicates potential for large impacts on native species. *Scientific Reports* 12:3603.

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. *Ecology and Evolution* 9:10241–10252.

Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P. 2012a. Dead or alive: Deformed wing virus and *Varroa destructor* reduce the life span of winter honeybees. *Applied and Environmental Microbiology* 78:981–987.

Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P. 2012b. Predictive markers of honey bee colony collapse. *PLoS ONE* 7:e32151.

Daniels TJ, Bekoff M. 1989. Feralization: The making of wild domestic animals. *Behavioural Processes* 19:79–94.

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? *Environmental Entomology* 46:737–746.

De la Rúa P, Jaffé R, Dall'Olio R, Muñoz I, Serrano J. 2009. Biodiversity, conservation and current threats to European honeybees. *Apidologie* 40:263–284.

de Miranda JR, Genersch E. 2010. Deformed wing virus. *Journal of Invertebrate Pathology* 103:48–61.

Deutscher Imkerbund. 2020. *Jahresbericht 2020*. Wachtberg-Villip.

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. *Proceedings of the National Academy of Sciences of the United States of America* 113:3203–3208.

Dick CW. 2001. Genetic rescue of remnant tropical trees by an alien pollinator. *Proceedings of the Royal Society B* 268:2391–2396.

Dicks LV., Breeze TD, Ngo HT, Senapathi D, An J, Aizen MA, Basu P, Buchori D, Galetto L, Garibaldi LA, Gemmill-Herren B, Howlett BG, Imperatriz-Fonseca VL, Johnson SD, Kovács-Hostyánszki A, Kwon YJ, Lattorff HMG, Lungharwo T, Seymour CL, Vanbergen AJ, Potts SG. 2021. A global-scale expert assessment of drivers and risks associated with pollinator decline. *Nature Ecology and Evolution* 5:1453–1461.

Dietemann V, Nazzi F, Martin SJ, Anderson DL, Locke B, Delaplane KS, Wauquiez Q, Tannahill C, Frey E, Ziegelmann B, Rosenkranz P, Ellis JD. 2013. Standard methods for *Varroa* research. *Journal of Apicultural Research* 52:1–54.

Dietemann V, Pirk CWW, Crewe R. 2009. Is there a need for conservation of honeybees in Africa? *Apidologie* 40:285–295.

Dogantzis KA, Tiwari T, Conflitti IM, Dey A, Patch HM, Muli EM, Garnery L, Whitfield CW, Stolle E, Alqarni AS, Allsopp MH, Zayed A. 2021. Thrice out of Asia and the adaptive radiation of the western honey bee. *Science Advances* 7:eabj2151.

Döke MA, Frazier M, Grozinger CM. 2015. Overwintering honey bees: Biology and management. *Current Opinion in Insect Science* 10:185–193.

Dolezal AG, Carrillo-Tripp J, Judd TM, Allen Miller W, Bonning BC, Toth AL. 2019. Interacting stressors matter: Diet quality and virus infection in honeybee health. *Royal Society Open Science* 6:181803.

Dubaić JB, Simonović S, Plećaš M, Stanisavljević L, Davidović S, Tanasković M, Ćetković A. 2021. Unprecedented density and persistence of feral honey bees in urban environments of a large SE-European city (Belgrade, Serbia). *Insects* 12:1127.

Dustmann JH, von der Ohe W. 1988. Einfluss von Kälteeinbrüchen auf die Frühjahrsentwicklung von Bienenvölkern (*Apis mellifera*). *Apidologie* 19:245–254.

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:e0216286.

Elbgami T, Kunin WE, Hughes WOH, Biesmeijer JC. 2014. The effect of proximity to a honeybee apiary on bumblebee colony fitness, development, and performance. *Apidologie* 45:504–513.

Evans JD. 2006. Beepath: An ordered quantitative-PCR array for exploring honey bee immunity and disease. *Journal of Invertebrate Pathology* 93:135–139.

Farrar CL. 1937. The influence of colony populations on honey production. *Journal of Agricultural Research* 54:945–954.

Faurot-Daniels C, Glenny W, Daughenbaugh KF, McMenamin AJ, Burkle LA, Flenniken ML. 2020. Longitudinal monitoring of honey bee colonies reveals dynamic nature of virus abundance and indicates a negative impact of Lake Sinai virus 2 on colony health. *PLoS ONE* 15:e0237544.

Floris I, Pusceddu M, Satta A. 2020. Birds and honey bees: A brief overview on this antagonistic relationship and its potential impact on beekeeping. *Redia* 103:65–67.

Fontana P, Costa C, Di Prisco G, Ruzzier E, Annoscia D, Battisti A, Caoduro G, Carpana E, Contessi A, Dal Lago A, Dall'olio R, de Cristofaro A, Felicioli A, Floris I, Fontanesi L, Gardi T, Lodesani M, Malagnini V, Manias L, Manino A, Marzi G, Massa B, Mutinelli F, Nazzi F, Pennacchio F, Porporato M, Stoppa G, Tormen N, Valentini M, Segrè A. 2018. Appeal for biodiversity protection of native honey bee subspecies of *Apis mellifera* in Italy (San michele all'Adige declaration). *Bulletin of Insectology* 71:257–271.

Food and Agriculture Organization of the United Nations. 2022.FAOSTAT Statistical Database. *Available at https://www.fao.org/faostat/en/#data/QCL*

Francis RM, Kryger P, Meixner M, Bouga M, Ivanova E, Andonov S, Berg S, Bienkowska M, Büchler R, Charistos L, Costa C, Dyrba W, Hatjina F, Panasiuk B, Pechhacker H, Kezić N, Korpela S, Conte Y Le, Uzunov A, Wilde J. 2014. The genetic origin of honey bee colonies used in the COLOSS Genotype-Environment Interactions Experiment: a comparison of methods. *Journal of Apicultural Research* 53:188–204.

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. *Journal of Economic Entomology* 107:508–515.

Fries I, Camazine S. 2001. Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. *Apidologie* 32:199–214.

Fries I, Lindström A, Korpela S. 2006. Vertical transmission of American foulbrood (*Paenibacillus larvae*) in honey bees (*Apis mellifera*). *Veterinary Microbiology* 114:269–274.

von Frisch K. 1967. *The dance language and orientation of bees*. Cambridge/Massachusetts: Harvard University Press.

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.

Gabel M, Scheiner R, Büchler R. 2023. Immediate and long-term effects of induced brood interruptions on the reproductive success of *Varroa destructor*. *Apidologie* 54:20.

Gainzarain JA, Fernández-García JM. 2013. Black woodpecker *Dryocopus martius* (L., 1758) recent range expansion leads to the coalescence of the two former distribution areas in northern Spain. *Munibe (Ciencias Naturales-Natur Zientziak)* 61:103–115.

Galizia CG, Eisenhardt D, Giurfa M (eds.). 2012. *Honeybee Neurobiology and Bahavior. A Tribute to Randolf Menzel*. Springer.

Gallai N, Salles JM, Settele J, Vaissière BE. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics* 68:810–821.

Ganser D, Albrecht M, Knop E. 2021. Wildflower strips enhance wild bee reproductive success. *Journal of Applied Ecology* 58:486–495.

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 Conservation and Diversity* 13:532–542.

Garibaldi LA, Steffan-dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalheiro LG. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* 339:1608–1611.

Garnery L, Cornuet J-M, Solignac M. 1992. Evolutionary history of the honey bee *Apis mellifera* inferred from mitochondrial DNA analysis. *Molecular Ecology* 1:145–154.

Geldmann J, González-Varo JP. 2018. Conserving honey bees does not help wildlife. *Science* 359:392–393.

Genersch E. 2010. American Foulbrood in honeybees and its causative agent, *Paenibacillus larvae*. *Journal of Invertebrate Pathology* 103:10–19.

Genersch E, von der Ohe W, Kaatz H, Schroeder A, Otten C, Büchler R, Berg S, Ritter W, Mühlen W, Gisder S, Meixner M, Liebig G, Rosenkranz P. 2010. The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. *Apidologie* 41:332–352.

Gering E, Incorvaia D, Henriksen R, Conner J, Getty T, Wright D. 2019. Getting back to nature: feralization in animals and plants. *Trends in Ecology and Evolution* 34:1137–1151.

Gerlach B, Dröschmeister R, Langgemach T, Borkenhagen K, Busch M, Hauswirth M, Heinicke T, Kamp J, Karthäuser J, König C, Markones N, Prior N, Trautmann S, Wahl J, Sudfeldt C. 2019. *Vögel in Deutschland – Übersichten zur Bestandssituation*. Münster: DDA, BfN, LAG VSW.

Gilley DC, Tarpy DR. 2005. Three mechanisms of queen elimination in swarming honey bee colonies. *Apidologie* 36:461–474.

Goodwin RM, Houten A Ten, Perry JH. 1994. Incidence of American foulbrood infections in feral honey bee colonies in New Zealand. *New Zealand Journal of Zoology* 21:285–287.

Gortázar C, Ferroglio E, Höfle U, Frölich K, Vicente J. 2007. Diseases shared between wildlife and livestock: A European perspective. *European Journal of Wildlife Research* 53:241–256.

Goulson D. 2010. *Bumblebees: behaviour, ecology, and conservation*. Oxford: Oxford University Press.

Goulson D, Hughes WOH. 2015. Mitigating the anthropogenic spread of bee parasites to protect wild pollinators. *Biological Conservation* 191:10–19.

Goulson D, Nicholls E, Botías C, Rotheray EL. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347:1255957.

Greenleaf SS, Kremen C. 2006. Wild bees enhance honey bees' pollination of hybrid sunflower. *Proceedings of the National Academy of Sciences of the United States of America* 103:13890–5.

Grindrod I, Martin SJ. 2021. Parallel evolution of *Varroa* resistance in honey bees: a common mechanism across continents? *Proceedings of the Royal Society B* 288:20211375.

Grüter C, Hayes L. 2022. Sociality is a key driver of foraging ranges in bees. *Current Biology* 32:5390–5397.

Guichoux E, Lagache L, Wagner S, Chaumeil P, Léger P, Lepais O, Lepoittevin C, Malausa T, Revardel E, Salin F, Petit RJ. 2011. Current trends in microsatellite genotyping. *Molecular Ecology Resources* 11:591–611.

Gunda B. 1968. Bee-hunting in the Carpathian area. *Acta Ethnographica Academiae Scientiarum Hungaricae* 17:1–62.

Habel JC, Samways MJ, Schmitt T. 2019. Mitigating the precipitous decline of terrestrial European insects: Requirements for a new strategy. *Biodiversity and Conservation*

28:1343–1360.

Harbo JR. 1986. Effect of population size on brood production, worker survival and honey gain in colonies of honeybees. *Journal of Apicultural Research* 25:22–29.

Hartig F. 2022.DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models. (R package version 0.4.5.) *Available at https://cran.r-project.org/package=DHARMa*

Henneken R, Helm S, Menzel A. 2012. Meteorological influences on swarm emergence in honey bees (Hymenoptera: Apidae) as detected by crowdsourcing. *Environmental Entomology* 41:1462–1465.

Henry M, Rodet G. 2018. Controlling the impact of the managed honeybee on wild bees in protected areas. *Scientific Reports* 8:9308.

Herrera CM. 2020. Gradual replacement of wild bees by honeybees in flowers of the Mediterranean Basin over the last 50 years. *Proceedings of the Royal Society B* 287:20192657.

Hood WM, Caron DM. 1997. Mammals. In: Morse RA, Flottum K eds. *Honey bee pests, predators and diseases*. Medina, Ohio: The A.I. Root Company, 357–399.

Hoppe A, Du M, Bernstein R, Tiesler F-K, Kärcher M, Bienefeld K. 2020. Substantial genetic progress in the international *Apis mellifera carnica* population since the implementation of genetic evaluation. *Insects* 11:768.

Hothorn T, Bretz F, Westfall P. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50:346–363.

Hothorn T, Hornik K, Wiel MA Van De, Zeileis A, Hothorn T, Hornik K, Wiel MA Van De, Zeileis A. 2016. A Lego system for conditional inference. *The American Statistician* 60:257–263.

Hung K-LJ, Kingston JM, Albrecht M, Holway DA, Kohn JR. 2018. The worldwide importance of honey bees as pollinators in natural habitats. *Proceedings of the Royal Society B* 285:20172140.

Ibisch PL, Hoffmann MT, Kreft S, Pe'er G, Kati V, Biber-Freudenberger L, DellaSala DA, Vale MM, Hobson PR, Selva N. 2016. A global map of roadless areas and their conservation status. *Science* 354:1423–1427.

Ilyasov RA, Kosarev MN, Neal A, Yumaguzhin FG. 2015. Burzyan bees in South Ural. *Bees for Development* 114:11–17.

Imdorf A, Ruoff K, Fluri P. 2008. Volksentwicklung bei der Honigbiene. *ALP forum* 68:1–88.

Iwasaki JM, Hogendoorn K. 2022. Mounting evidence that managed and introduced bees have negative impacts on wild bees: an updated review. *Current Research in Insect Science* 2:100043.

Jaffé R, Dietemann V, Allsopp MH, Costa C, Crewe RM, Dall'olio R, De la Rúa P, El-Niweiri M a a, Fries I, Kezic N, Meusel MS, Paxton RJ, Shaibi T, Stolle E, Moritz RF a. 2010. Estimating the density of honeybee colonies across their natural range to fill the gap in pollinator decline censuses. *Conservation Biology* 24:583–593.

Jedrzejewski W, Zalewski A, Jedrzejewska B. 1993. Foraging by pine marten *Martes martes* in relation to food resources in Bialowieza National Park, Poland. *Acta Theriologica* 38:405–426.

Jensen AB, Palmer KA, Chaline N, Raine NE, Tofilski A, Martin SJ, Pedersen B V, Boomsma JJ, Ratnieks FLW. 2005. Quantifying honey bee mating range and isolation in semi-isolated valleys by DNA microsatellite paternity analysis. 6:527–537.

Johannesen J, Wöhl S, Berg S, Otten C. 2022. Annual fluctuations in winter colony losses of *Apis mellifera* L. are predicted by honey flow dynamics of the preceding year. *Insects* 13:829.

Johnson K, Nilsson SG, Tjernberg M. 1993. Characteristics and utilization of old Black Woodpecker *Dryocopus martius* holes by hole-nesting species. *IBIS* 135:410–416.

Jones OR, Wang J. 2010. COLONY: A program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources* 10:551–555.

Kauhausen-Keller D, Keller R. 1994. Morphometrical control of pure race breeding in the honeybee (*Apis mellifera* L). *Apidologie* 25:133–143.

Kearns CA, Inouye DW, Waser N. 1998. Endangered mutualism: The conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics* 29:83–112.

Klein a.-M, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham S a, Kremen C, Tscharntke T. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B* 274:303–313.

Koeniger G, Koeniger N. 2014. Paarungsbiologie bei der Honigbiene. In: *Paarungsbiologie und Paarungskontrolle bei der Honigbiene*. Herten: Buschhausen Druck- und Verlagshaus, 1–148.

Koeniger N, Koeniger G, Pechhacker H. 2005. The nearer the better? Drones (*Apis mellifera*) prefer nearer drone congregation areas. *Insectes Sociaux* 52:31–35.

Kohl PL, D'Alvise P, Rutschmann B, Roth S, Remter F, Steffan-Dewenter I, Hasselmann M. 2022. Reduced parasite burden in feral honeybee colonies. *bioRxiv*:2022.07.18.500457.

Kohl PL, Grziwotz F, Telschow A, Kurtz J, Mühlen W. 2015. The neglected gender – drone production in naturally nesting and beekeeper-managed honeybee colonies (*Apis mellifera*). In: *62. Tagung der Arbeitsgemeinschaft der Institute für Bienenforschung e.V.* 67.

Kohl PL, Rutschmann B. 2018. The neglected bee trees: European beech forests as a home for feral honey bee colonies. *PeerJ* 6:e4602.

Kohl PL, Rutschmann B, Steffan-Dewenter I. 2022. Population demography of feral honeybee colonies in central European forests. *Royal Society Open Science* 9:220565.

Kosiński Z, Bilińska E, Dereziński J, Jeleń J, Kempa M. 2010. The Black Woodpecker (*Dryocopus martius*) and the European Beech (*Fagus sylvatica*) as keystone species for the Stock Dove (*Columba oenas*) in western Poland. *Ornis Polonica* 51:1–13.

Kosiński Z, Walczak Ł. 2019. Does cavity reuse affect timing of reproduction and fledgling

success in the Black Woodpecker? *Journal of Ornithology* 160:79–89.

Kraus B, Page RER. 1995. Effect of *Varroa jacobsoni* (Mesostigmata: Varroidae) on feral *Apis mellifera* (Hymenoptera: Apidae) in California. *Environmental Entomology* 24:1473–1480.

Krebs CJ. 2014. *Ecology: the experimental analysis of distribution and abundance*. Pearson Education Limited.

Küster H. 1998. *Geschichte des Waldes von der Urzeit bis zur Gegenwart*. München: C. H. Beck.

Lang U, Albouy V, Zewen C. 2022. Comparative monitoring of free-living honey bee colonies in three Western European regions. *Natural Bee Husbandry Magazine* 23:23–31.

Latham RE, Ricklefs RE. 1993. Continental comparisons of temperate-zone tree species diversity. In: Ricklefs RE, Schluter D eds. *Species diversity in ecological communities: historical and geographical perspectives*. Chicago: The University of Chicago Press, 294–314.

Lecocq T. 2018. Insects: The disregarded domestication histories. In: Teletchea F ed. *Animal Domestication*. London: IntechOpen.

Lee PC, Winston ML. 1987. Effects of reproductive timing and colony size on the survival, offspring colony size and drone production in the honey bee *Apis mellifera*. *Ecological Entomology* 12:187–195.

Lenth R V. 2022.emmeans: Estimated marginal means, aka least-squares means (R package version 1.7.3). *Available at https://cran.r-project.org/package=emmeans*

Leonhardt SD, Gallai N, Garibaldi LA, Kuhlmann M, Klein A-M. 2013. Economic gain, stability of pollination and bee diversity decrease from southern to northern Europe. *Basic and Applied Ecology* 14:461–471.

Lindauer M. 1955. Schwarmbienen auf Wohnungssuche. *Zeitschrift für vergleichende Physiologie* 37:263–324.

Lindenmayer D, Crane M, Blanchard W, Okada S, Montague-Drake R. 2016. Do nest boxes in restored woodlands promote the conservation of hollow-dependent fauna? *Restoration Ecology* 24:244–251.

Lindenmayer DB, Laurance WF. 2017. The ecology, distribution, conservation and management of large old trees. *Biological Reviews* 92:1434–1458.

Lindenmayer DB, Laurance WF, Franklin JF. 2012. Global decline in large old trees. *Science* 338:1305–1306.

Lindström SAM, Herbertsson L, Rundlöf M, Bommarco R, Smith HG. 2016. Experimental evidence that honeybees depress wild insect densities in a flowering crop. *Proceedings of the Royal Society B* 283:20161641.

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.

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. *Applied and Environmental Microbiology* 78:227–235.

Lodesani M, Costa C. 2003. Bee breeding and genetics in Europe. *Bee World* 84:69–85.

Loftus JC, Smith ML, Seeley TD. 2016. How honey bee colonies survive in the wild: testing the importance of small nests and frequent swarming. *PLoS ONE* 11:e0150362.

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

Lotka JA. 1925. The measure of net fertility. *Journal of the Washington Academy of Sciences* 15:469–472.

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.

Louveaux J, Albisetti M, Delangue M, Theurkauff M. 1966. Les modalités de l'adaptation des abeilles (*Apis mellifica* L.) au milieu naturel. *Annales de l'Abeille* 9:323–350.

Mallinger RE, Gaines-Day HR, Gratton C. 2017. Do managed bees have negative effects on wild bees?: A systematic review of the literature. *PLoS ONE* 12:e0189268.

Manning R, Lancaster K, Rutkay A, Eaton L. 2007. Survey of feral honey bee (*Apis mellifera*) colonies for *Nosema apis* in Western Australia. *Australian Journal of Experimental Agriculture* 47:883–886.

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. *International Journal for Parasitology: Parasites and Wildlife* 18:184–193.

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. *Letters in Applied Microbiology* 50:603–610.

Mashilingi SK, Zhang H, Garibaldi LA, An J. 2022. Honeybees are far too insufficient to supply optimum pollination services in agricultural systems worldwide. *Agriculture, Ecosystems & Environment* 335:108003.

Matschiner M, Salzburger W. 2009. TANDEM: Integrating automated allele binning into genetics and genomics workflows. *Bioinformatics* 25:1982–1983.

Mattila HR, Harris JL, Otis GW. 2001. Timing of production of winter bees in honey bee (*Apis mellifera*) colonies. *Insectes Sociaux* 48:88–93.

McGlynn TP. 2012. The ecology of nest movement in social insects. *Annual Review of Entomology* 57:291–308.

McMahon DP, Natsopoulou ME, Doublet V, Fürst M, Weging S, Brown MJFF, Gogol-Döring A, Paxton RJ. 2016. Elevated virulence of an emerging viral genotype as a driver of honeybee loss. *Proceedings of the Royal Society B* 283:20160811.

Meixner MD, Kryger P, Costa C. 2015. Effects of genotype, environment, and their interactions on honey bee health in Europe. *Current Opinion in Insect Science* 10:177–184.

Mikheyev AS, Tin MMY, Arora J, Seeley T. 2016. Museum samples reveal rapid evolution by wild honey bees exposed to a novel parasite. *Nature Communications* 6:7991.

Millon A, Lambin X, Devillard S, Schaub M. 2019. Quantifying the contribution of immigration to population dynamics: a review of methods, evidence and perspectives in birds and mammals. *Biological Reviews* 94:2049–2067.

Mittl S. 2017. *Apis mellifera* und das Bundesnaturschutzgesetz (BNatSchG) der BRD – Ist die Art *Apis mellifera* (Westliche Honigbiene) ein Wildtier und welche Folgen hätte das für Gesetzgebung und Artenschutz? (2. überarbeitete Fassung). *Available at https://bienen-dialoge.de/apis-mellifera-und-das-bundesnaturschutzgesetz/* (accessed May 11, 2023).

Mittl S. 2019.Die Reinzuchtbelegstellen der Stämme der Dunklen Biene *Apis mellifera mellifera* und der Kärntner Biene *Apis mellifera carnica* zwischen 1934 und 1945 und die Geschichte der Zucht in dieser Zeit mit Ausblick in die Zeit danach – Teil 1. *Available at https://bienen-dialoge.de/die-reinzuchtbelegstellen-und-die-zucht-von-apis-mellifera-mellifera-und-apis-mellifera-carnica-zwischen-1934-und-1945-mit-ausblick-auf-die-zeit-danach-teil-1/* (accessed May 11, 2023).

Moritz RFA. 1991. The limitations of biometric control on pure race breeding in *Apis mellifera*. *Journal of Apicultural Research* 30:54–59.

Moritz RFA, Crewe RM. 2018. *The dark side of the hive: the evolution of the imperfect honeybee*. New York: Oxford University Press.

Moritz RFA, Erler S. 2016. Lost colonies found in a data mine: Global honey trade but not pests or pesticides as a major cause of regional honeybee colony declines. *Agriculture, Ecosystems and Environment* 216:44–50.

Moritz RFA, Härtel S, Neumann P. 2005. Global invasions of the western honeybee (*Apis mellifera*) and the consequences for biodiversity. *Écoscience* 12:289–301.

Moro A, Beaurepaire A, Olio RD, Rogenstein S, Blacquière T, Dahle B, Miranda JR De, Dietemann V, Locke B, María R, Luna L, Conte Y Le, Neumann P. 2021. Using citizen science to scout honey bee colonies that naturally survive *Varroa destructor* infestations. *Insects* 12:536.

Morse RA. 1991. Honeybees forever. *Trends in Ecology and Evolution* 6:337–338.

Morse RA, Flottum K (eds.). 1997. *Honey bee pests, predators and diseases*. Medina, Ohio: The A. I. Root Company.

Mysterud A, Rolandsen CM. 2019. Fencing for wildlife disease control. *Journal of Applied Ecology* 56:519–525.

Neumann P, Blacquière T. 2017. The Darwin cure for apiculture? Natural selection and managed honey bee health. *Evolutionary Applications* 10:226–230.

Neumannu P, Praaghc JP Van, Moritzux RFA, Dustmannc JH. 1999. Testing reliability of a potential island mating apiary using DNA microsatellites. *Apidologie* 30:257–276.

Newton I. 1994. The role of nest sites in limiting the numbers of hole-nesting birds: a review. *Biological Conservation* 70:265–276.

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.

Norton AM, Remnant EJ, Tom J, Buchmann G, Blacquiere T, Beekman M. 2021. Adaptation to vector-based transmission in a honeybee virus. *Journal of Animal Ecology* 90:2254–2267.

Oberreiter H, Dünser A, Schlagbauer J, Brodschneider R. 2021. The wilderness area Dürrenstein – a habitat for feral honey bee colonies? *Entomologica Austriaca* 28:25–42.

Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J. 2022.vegan: Community ecology package. (R package version 2.6-4.) *Available at https://cran.r-project.org/package=vegan*

Oldroyd BP, Lawler SH, Crozier RH. 1994. Do feral honey bees (*Apis mellifera*) and regent parrots (*Polytelis anthopeplus*) compete for nest sites? *Austral Journal of Ecology* 19:444–450.

Oldroyd BP, Thexton EG, Lawler SH, Crozier RH. 1997. Population demography of Australian feral bees (*Apis mellifera*). *Oecologia* 111:381–387.

Oleksa A, Gawroński R, Tofilski A. 2013. Rural avenues as a refuge for feral honey bee population. *Journal of Insect Conservation* 17:465–472.

Pacífico EC, Efstathion CA, Filadelfo T, Horsburgh R, Cunha RA, Paschotto FR, Denes F V, Gilardi J, Tella JL. 2020. Experimental removal of invasive Africanized honey bees increased breeding population size of the endangered Lear's macaw. *Pest Management Science* 76:4141–4149.

Palmer C. 2011. The moral relevance of the distinction between domesticated and wild animals. In: Beauchamp TL, Frey RG eds. *The Oxford Handbook of Animal Ethics*. New York: Oxford University Press.

Panziera D, Requier F, Chantawannakul P, Pirk CWW, Blacquière T. 2022. The diversity decline in wild and managed honey bee populations urges for an integrated conservation approach. *Frontiers in Ecology and Evolution* 10:767950.

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. *Journal of Zoo and Wildlife Medicine* 45:219–227.

Parreño MA, Alaux C, Brunet JL, Buydens L, Filipiak M, Henry M, Keller A, Klein AM, Kuhlmann M, Leroy C, Meeus I, Palmer-Young E, Piot N, Requier F, Ruedenauer F, Smagghe G, Stevenson PC, Leonhardt SD. 2022. Critical links between biodiversity and health in wild bee conservation. *Trends in Ecology and Evolution* 37:309–321.

Patenković A, Tanasković M, Erić P, Erić K, Mihajlović M, Stanisavljević L, Davidović S. 2022. Urban ecosystem drives genetic diversity in feral honey bee. *Scientific Reports* 12:17692.

Paton DC. 1996. *Overview of feral and managed honeybees in Australia: distribution, abundance, extent of interactions with native biota, evidence of impacts and future*

research. Canberra: Australian Nature Conservation Agency.

Paxton RJ, Schäfer MO, Nazzi F, Zanni V, Annoscia D, Marroni F, Bigot D, Laws-Quinn ER, Panziera D, Jenkins C, Shafiey H. 2022. Epidemiology of a major honey bee pathogen, deformed wing virus: potential worldwide replacement of genotype A by genotype B. *International Journal for Parasitology: Parasites and Wildlife* 18:157–171.

Peck DT. 2018. Behavioral mechanisms underlying parasite spread and host survival in the *Varroa destructor*/*Apis mellifera* parasite/host system. Cornell University.

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.

Pew J, Muir PH, Wang J, Frasier TR. 2015. related: An R package for analysing pairwise relatedness from codominant molecular markers. *Molecular Ecology Resources* 15:557–561.

Phiri BJ, Fèvre D, Hidano A. 2022. Upward global trends in managed honey bee colonies and production: a six-decade viewpoint, 1961-2017. *Scientific Reports* 12:21298.

Piot N, Schweiger O, Meeus I, Yañez O, Straub L, Villamar-Bouza L, De la Rúa P, Jara L, Ruiz C, Malmstrøm M, Mustafa S, Nielsen A, Mänd M, Karise R, Tlak-Gajger I, Özgör E, Keskin N, Diévart V, Dalmon A, Gajda A, Neumann P, Smagghe G, Graystock P, Radzevičiūtė R, Paxton RJ, de Miranda JR. 2022. Honey bees and climate explain viral prevalence in wild bee communities on a continental scale. *Scientific Reports* 12:1904.

Pirk CWW, Crewe RM, Moritz RFA. 2017. Risks and benefits of the biological interface between managed and wild bee pollinators. *Functional Ecology* 31:47–55.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. 2010a. Global pollinator declines: Trends, impacts and drivers. *Trends in Ecology and Evolution* 25:345–353.

Potts SG, Roberts SPM, Dean R, Marris G, Brown M, Jones R, Settele J. 2010b. Declines of managed honey bees and beekeepers in Europe. *Journal of Apicultural Research* 49:15–22.

QGIS Development Team. 2021.QGIS Geographic Information System. *Available at https://www.qgis.org*

R Core Team. 2022.R: A Language and Environment for Statistical Computing. *Available at https://www.r-project.org/*

Radcliffe RW, Seeley TD. 2018. Deep forest bee hunting: a novel method for finding wild colonies of honey bees in old-growth forests. *American Bee Journal* 158:871–877.

Rangel J, Giresi M, Pinto MA, Baum KA, Rubink WL, Coulson RN, Johnston JS. 2016. Africanization of a feral honey bee (*Apis mellifera*) population in South Texas: Does a decade make a difference? *Ecology and Evolution* 6:2158–2169.

Ratnieks FW, Nowakowski J. 1989. Honeybee swarms accept bait hives contaminated with American foulbrood disease. *Ecological Entomology* 14:475–478.

Ratnieks FLW, Shackleton K. 2015. Does the waggle dance help honey bees to forage at greater

distances than expected for their body size? *Frontiers in Ecology and Evolution* 3:31.

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:e72443.

Ravoet J, De Smet L, Meeus I, Smagghe G, Wenseleers T, de Graaf DC. 2014. Widespread occurrence of honey bee pathogens in solitary bees. *Journal of Invertebrate Pathology* 122:55–58.

Reinsch A. 1979. Bienen und Hornissen als Nistplatzkonkurrenten der Hohltaube *Columba oenas*. *Ornithologischer Anzeiger* 18:190.

Reinsch N, Schuster H, Bienefeld K, Pirchner F. 1991. Morphologischer Vergleich von Völkern der "Landbiene" in Niedersachsen mit typischer *Apis mellifera carnica* und *Apis mellifera mellifera*. *Apidologie* 22:75–80.

Remm J, Lõhmus A. 2011. Tree cavities in forests – the broad distribution pattern of a keystone structure for biodiversity. *Forest Ecology and Management* 262:579–585.

Requier F, Garnery L, Kohl PL, Njovu HK, Pirk CWW, Crewe RM, Steffan-dewenter I. 2019a. The conservation of native honey bees is crucial. *Trends in Ecology & Evolution* 34:789–798.

Requier F, Odoux JF, Henry M, Bretagnolle V. 2017. The carry-over effects of pollen shortage decrease the survival of honeybee colonies in farmlands. *Journal of Applied Ecology* 54:1161–1170.

Requier F, Odoux JF, Tamic T, Moreau N, Henry M, Decourtye A, Bretagnolle V. 2015. Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. *Ecological Applications* 25:881–890.

Requier F, Paillet Y, Laroche F, Rutschmann B, Zhang J, Lombardi F, Svoboda M, Steffan-Dewenter I. 2020. Contribution of European forests to safeguard wild honeybee populations. *Conservation Letters* 13:e12693.

Requier F, Rome Q, Chiron G, Decante D, Marion S, Menard M, Muller F, Villemant C, Henry M. 2019b. Predation of the invasive Asian hornet affects foraging activity and survival probability of honey bees in Western Europe. *Journal of Pest Science* 92:567–578.

Ribière M, Olivier V, Blanchard P. 2010. Chronic bee paralysis: A disease and a virus like no other? *Journal of Invertebrate Pathology* 103:120–131.

Rinderer TE, Baxter JR. 1978. Effect of empty comb on hoarding behavior and honey production of the honey bee. *Journal of Economic Entomology* 71:757–759.

Roberts JMK, Simbiken N, Dale C, Armstrong J, Anderson DL. 2020. Tolerance of honey bees to *Varroa* mite in the absence of deformed wing virus. *Viruses* 12:575.

Rollin O, Garibaldi LA. 2019. Impacts of honeybee density on crop yield: A meta-analysis. *Journal of Applied Ecology* 56:1152–1163.

Rosenkranz P, Aumeier P, Ziegelmann B. 2010. Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology* 103:96–119.

Rothenbuhler WC. 1958. Genetics and breeding of the honey bee. *Annu. Rev. Entomol.* 3:161–180.

Rowell GA, Taylor OR j., Long-Rowell MA. 1992. Spatial dynamics of the honey bee mating system (*Apis mellifera* L.). *Journal of the Kansas Entomological Society* 65:218–222.

Royce LA, Rossignol PA, Burgett DM, Stringer BA. 1991. Reduction of tracheal mite parasitism of honey bees by swarming. *Philosophical Transactions of the Royal Society B* 331:123–129.

Rutschmann B, Kohl PL, Machado A, Steffan-Dewenter I. 2022. Semi-natural habitats promote winter survival of wild-living honeybees in an agricultural landscape. *Biological Conservation* 266:109450.

Rutschmann B, Kohl PL, Steffan-Dewenter I. 2023. Foraging distances, habitat preferences and seasonal colony performance of honeybees in Central European forest landscapes. *Journal of Applied Ecology* (Early View).

Ruttner F. 1988a. *Biogeography and Taxonomy of Honeybees*. Berlin, Heidelberg: Springer.

Ruttner F. 1988b. *Breeding techniques and selection for breeding of the honey bee*. Munich: The British Isles Bee Breeders Association by arrangement with Ehrenwirth Verlag.

Ruttner F. 1992. *Naturgeschichte der Honigbienen*. München: Ehrenwirth.

Ruttner F, Milner E, Dews JE. 1990. *The dark european honey bee Apis mellifera mellifera Linnaeus 1758*. British Isles Bee Breeders Association.

Saunders ME, Goodwin EK, Santos KCBS, Sonter CA, Rader R. 2021. Cavity occupancy by wild honey bees: need for evidence of ecological impacts. *Frontiers in Ecology and the Environment* 19:349–354.

Scheper J, Reemer M, Van Kats R, Ozinga WA, Van Der Linden GTJ, Schaminée JHJ, Siepel H, Kleijn D. 2014. Museum specimens reveal loss of pollen host plants as key factor driving wild bee decline in the Netherlands. *Proceedings of the National Academy of Sciences of the United States of America* 111:17552–17557.

Schirach AG. 1774. *Wald-Bienenzucht, Nach ihren großen Vortheilen, leichten Anlegung und Abwartung*. Breslau: Firma Wilhelm Gottlieb Korn.

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

Schmidt M, Meyer P, Mölder A, Hondong H. 2016. Neu- oder Wiederausbreitung? Die Arealausweitung des Schwarzspechts in Nordwestdeutschland am Ende des 19. Jahrhunderts und ihre Ursachen. *Forstarchiv* 87:135–151.

Schommer TJ, Woolever MM. 2008. *A review of disease related conflicts between domestic sheep and goats and bighorn sheep*. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

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. *Journal of Eukaryotic Microbiology* 62:567–583.

Seeley T. 1977. Measurement of nest cavity volume by the honey bee (*Apis mellifera*). *Behavioral Ecology and Sociobiology* 2:201–227.

Seeley TD. 1978. Life history strategy of the honey bee, *Apis mellifera*. *Oecologia* 32:109–118.

Seeley TD. 1985. *Honeybee Ecology: A Study of Adaptation in Social Life*. Princeton, New Jersey: Princeton University Press.

Seeley TD. 1995. *The Wisdom of the Hive: The Social Physiology of Honey Bee Colonies"*. Cambridge/Massachusetts: Harvard University Press.

Seeley TD. 2002. The effect of drone comb on a honey bee colony' s production of honey. *Apidologie* 33:75–86.

Seeley TD. 2007. Honey bees of the Arnot Forest: a population of feral colonies persisting with *Varroa destructor* in the northeastern United States. *Apidologie* 38:19–29.

Seeley TD. 2010. *Honeybee Democracy*. Princeton, New Jersey: Princeton University Press.

Seeley TD. 2016. *Following the wild bees: The craft and science of bee hunting*. Princeton, New Jersey: Princeton University Press.

Seeley TD. 2017. Life-history traits of wild honey bee colonies living in forests around Ithaca, NY, USA. *Apidologie* 48:743–754.

Seeley TD. 2019. *The Lives of Bees. The Untold Story of the Honey Bee in the Wild*. Princeton and Oxford: Princeton University Press.

Seeley TD, Morse RA. 1976. The nest of the honey bee (*Apis mellifera* L.). *Insectes Sociaux* 23:495–512.

Seeley TD, Morse RA. 1977. Dispersal behavior of honey bee swarms. *Psyche* 84:199–209.

Seeley TD, Morse RA. 1978. Nest site selection by the honey bee, *Apis mellifera*. *Insectes Sociaux* 25:323–337.

Seeley TD, Morse RA, Nowogrodzki R. 1989. *Bait hives for honey bees*. Cornell Cooperative Extension.

Seeley TD, Smith ML. 2015. Crowding honeybee colonies in apiaries can increase their vulnerability to the deadly ectoparasite *Varroa destructor*. *Apidologie* 46:716–727.

Seeley TD, Tarpy DR, Griffin SR, Carcione A, Delaney D a. 2015. A survivor population of wild colonies of European honeybees in the northeastern United States: investigating its genetic structure. *Apidologie* 46:654–666.

Seeley TD, Visscher PK. 1985. Survival of honeybees in cold climates: the critical timing of colony growth and reproduction. *Ecological Entomology* 10:81–88.

Seibold S, Gossner MM, Simons NK, Blüthgen N, Müller J, Ambarlı D, Ammer C, Bauhus J, Fischer M, Habel JC, Linsenmair KE, Nauss T, Penone C, Prati D, Schall P, Schulze ED, Vogt J, Wöllauer S, Weisser WW. 2019. Arthropod decline in grasslands and forests is associated with landscape-level drivers. *Nature* 574:671–674.

Shaibi T, Lattorff HMG, Moritz RFA. 2008. A microsatellite DNA toolkit for studying

population structure in *Apis mellifera*. *Molecular Ecology Resources* 8:1034–1036.

Sikora LG. 2009. *Erfassen von Schwarzspecht-Höhlenbäumen im Biosphärengebiet Schwäbische Alb*. Deutsche Wildtier Stiftung.

Sikora LG, Schnitt D, Kinser A. 2016. *Folgeuntersuchung von Schwarzspecht-Höhlenbäumen im Biosphärengebiet Schwäbische Alb*. Deutsche Wildtier Stiftung.

Simone-Finstrom M, Spivak M. 2010. Propolis and bee health: the natural history and significance of resin use by honey bees. *Apidologie* 41:295–311.

Smith ML, Koenig PA, Peters JM. 2017. The cues of colony size: How honey bees sense that their colony is large enough to begin to invest in reproduction. *Journal of Experimental Biology* 220:1597–1605.

Soland-Reckeweg G, Heckel G, Neumann P, Fluri P, Excoffier L. 2009. Gene flow in admixed populations and implications for the conservation of the Western honeybee, *Apis mellifera*. *Journal of Insect Conservation* 13:317–328.

Sponsler D, Johnson R. 2015. Honey bee success predicted by landscape composition in Ohio, USA. *PeerJ* 3:e838.

Stanley DA, Msweli SM, Johnson SD. 2020. Native honeybees as flower visitors and pollinators in wild plant communities in a biodiversity hotspot. *Ecosphere* 11:e02957.

Steffan-Dewenter I, Kuhn A. 2003. Honeybee foraging in differentially structured landscapes. *Proceedings of the Royal Society B* 270:569–575.

Stephens M, Smith NJ, Donnelly P. 2001. A new statistical method for haplotype reconstruction from population data. *The American Journal of Human Genetics* 68:978-989.

Stevenson M, Sergeant E, Nunes T, Heuer C, Marshall J, Sanchez J, Thornton R, Reiczigel J, Robison-Cox J, Sebastiani P, Solymos P, Yoshida K, Jones G, Pirikahu S, Firestone S, Kyle R, Popp J, Jay M, Reynard C, Cheung A, Singanallur N, Szabo A, Rabiee A. 2022.epiR: tools for the analysis of epidemiological data (R package version 2.0.46). *Available at https://cran.r-project.org/package=epiR*

Stoeckhert FK. 1933. *Die Bienen Frankens (Hym. Apid.): eine ökologisch-tiergeographische Untersuchung*. Berlin: R. Friedländer & Sohn.

Stoeckhert FK. 1954. Fauna Apoideorum Germaniae (Nachträge und Ergänzungen zu F. K. Stoeckhert, Die Bienen Frankens, Deutsche Entomologische Zeitschrift 1932, Beiheft, Berlin 1933). *Abhandlungen der Bayerischen Akademie der Wissenschaften Mathematisch-naturwissenschaftliche Klasse* Neue Folge:69.

Strobl V, Yañez O, Straub L, Albrecht M, Neumann P. 2019. Trypanosomatid parasites infecting managed honeybees and wild solitary bees. *International Journal for Parasitology* 49:605–613.

Taylor MA, Goodwin RM, McBrydie HM, Cox HM. 2007. Destroying managed and feral honey bee (*Apis mellifera*) colonies to eradicate honey bee pests. *New Zealand Journal of Crop and Horticultural Science* 35:313–323.

Tehel A, Brown MJF, Paxton RJ. 2016. Impact of managed honey bee viruses on wild bees. *Current Opinion in Virology* 19:16–22.

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. *Proceedings of the Royal Society B* 289:20212255.

Themudo GE, Rey-Iglesia A, Robles Tascón L, Jensen AB, da Fonseca RR, Campos PF. 2020. Declining genetic diversity of European honeybees along the twentieth century. *Scientific Reports* 10:110520.

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.

Thompson CE, Biesmeijer JC, Allnutt TR, Pietravalle S, Budge GE. 2014. Parasite pressures on feral honey bees (*Apis mellifera* sp.). *PloS one* 9:e105164.

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 in Parasitology* 36:592–606.

Uribe MML, Appler RH, Youngsteadt E, Dunn RR, Frank SD, Tarpy DR. 2017. Higher immunocompetence is associated with higher genetic diversity in feral honey bee colonies (*Apis mellifera*). *Conservation Genetics* 18:659–666.

vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen Y, Underwood R, Tarpy DR, Pettis JS. 2009. Colony collapse disorder: A descriptive study. *PLoS ONE* 4:e6481.

VanEngelsdorp D, Meixner MD. 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *Journal of invertebrate pathology* 103:S80-95.

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. *Annals of the Entomological Society of America* 101:867–871.

Visick O, Ratnieks F. 2022. Density of wild-living honey bee, *Apis mellifera*, colonies worldwide: A review. *Authorea Preprints*.

Visick OD, Ratnieks FLW. 2023. Ancient, veteran and other listed trees as nest sites for wild honey bee, *Apis mellifera*, colonies. *ResearchSquare*.

Visscher PK, Morse RA, Seeley TD. 1985. Honey bees choosing a home prefer previously occupied cavities. *Insectes Sociaux* 32:217–220.

Visscher PK, Seeley TD. 1982. Foraging strategy of honeybee colonies in a temperate deciduous forest. *Ecology* 63:1790–1801.

Visscher P, Seeley T. 1989. Bee-lining as a research technique in ecological studies of honey bees. *American Bee Journal* 129:536–539.

Wagner DL. 2020. Insect declines in the anthropocene. *Annual Review of Entomology* 65:457–480.

Wang J. 2007. Triadic IBD coefficients and applications to estimating pairwise relatedness.

Genetical Research 89:135–153.

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. *International Journal of Applied Earth Observation and Geoinformation* 88:102065.

Weinstock GM. 2006. Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature* 443:931–49.

White TCR. 2008. The role of food, weather and climate in limiting the abundance of animals. *Biological Reviews* 83:227–248.

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

Wignall VR, Brolly M, Uthoff C, Norton KE, Chipperfield HM, Balfour NJ, Ratnieks FLW. 2020. Exploitative competition and displacement mediated by eusocial bees: experimental evidence in a wild pollinator community. *Behavioral Ecology and Sociobiology* 74:152.

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* 351:594–597.

Williams JL. 1997. Insects: Lepidoptera (moths). In: Morse RA, Flottum K eds. *Honey bee pests, predators and diseases*. Medina, Ohio: The A. I. Root Company, 119–142.

Williams IH. 2002. Insect pollination and crop production: a european perspective. In: Kevan P, Imperatriz-Fonseca VL eds. *Pollinating bees – the conservation link between agriculture and nature*. Brasília: Ministry of Environment, 59–65.

Winston ML. 1980. Swarming, afterswarming, and reproductive rate of unmanaged honeybee colonies (*Apis mellifera*). *Insectes Sociaux* 27:391–398.

Winston ML. 1991. *The biology of the honey bee*. Harvard University Press.

Winston ML. 1992. The biology and management of africanized honey bees. *Annual Review of Entomology* 37:173–193.

Winston ML, Taylor OR, Otis GW. 1983. Some differences between temperate European and tropical African and South American honeybees. *Bee World* 64:12–21.

Woodgate JL, Makinson JC, Rossi N, Lim KS, Reynolds AM, Rawlings CJ, Chittka L. 2021. Harmonic radar tracking reveals that honeybee drones navigate between multiple aerial leks. *iScience* 24:102499.

Youngsteadt E, Appler RH, López-Uribe MM, Tarpy DR, Frank SD. 2015. Urbanization increases pathogen pressure on feral and managed honey bees. *PLoS ONE* 10:e0142031.

Zahner V, Bauer R, Kaphegyi TAM. 2017. Are black woodpecker (*Dryocopus martius*) tree cavities in temperate beech (*Fagus sylvatica*) forests an answer to depredation risk? *Journal of Ornithology* 158:1073–1079.

Zahner V, Sikora L, Pasinelli G. 2012. Heart rot as a key factor for cavity tree selection in the black woodpecker. *Forest Ecology and Management* 271:98–103.

Zander E. 1944. *Handbuch der Bienenkunde in Einzeldarstellungen V. Die Zucht der Biene*.

Stuttgart: Verlag Eugen Ulmer.

Zander E, Weiss K. 1964. *Das Leben der Bienen*. Stuttgart: Eugen Ulmer.

List of publications

Publications and submitted manuscripts as part of this doctoral thesis:

Kohl PL, Rutschmann B, Sikora LG, Wimmer N, Zahner V, D'Alvise P, Hasselmann M, Steffan-Dewenter I (2023). Parasites, depredators and limited resources as potential drivers of winter mortality of feral honeybee colonies in German forests. (submitted)

Kohl PL, D'Alvise P, Rutschmann B, Roth S, Remter F, Steffan-Dewenter I, Hasselmann M (2022). Reduced parasite burden in feral honeybee colonies. *bioRxiv* 2022.07.18.500457 (preprint)

Kohl PL, Rutschmann B, Steffan-Dewenter I (2022). Population demography of feral honeybee colonies in central European forests. *Royal Society Open Science* 9:220565.

Other scientific publications of the candidate:

Kohl PL, Rutschmann B, Brockmann A (in press). Dance communication of giant honeybees. In: Abrol DP (ed). *Role of giant honeybees in natural and agricultural systems*. CRC Press.

Rutschmann B, **Kohl PL**, Steffan-Dewenter I (2023). Foraging distances, habitat preferences and seasonal colony performance of honeybees in Central European forest landscapes. *Journal of Applied Ecology*. (Early View)

Kohl PL, Steffan-Dewenter I (2022). Nectar robbing rather than pollinator availability constrains reproduction of a bee-flowered plant at high elevations. *Ecosphere* 13:e4077.

Rutschmann B, **Kohl PL**, Machado A, Steffan-Dewenter I (2022). Semi-natural habitats promote winter survival of wild-living honeybees in an agricultural landscape. *Biological Conservation* 266:109450.

George EA, Thulasi N, **Kohl PL**, Suresh S, Rutschmann B, Brockmann A (2021). Distance estimation by Asian honey bees in two visually different landscapes. *Journal of Experimental Biology* 224:jeb242404.

Kohl PL, Rutschmann B (2021). Honey bees communicate distance via non-linear waggle duration functions. *PeerJ* 9:e11187.

Young AM, **Kohl PL**, Rutschmann B, Steffan-Dewenter I, Brockmann A, Dyer FC (2021). Temporal and spatial foraging patterns of three Asian honey bee species in Bangalore, India. *Apidologie* 52:503–523.

Kohl PL, Thulasi N, Rutschmann B, George EA, Steffan-Dewenter I, Brockmann A (2020). Adaptive evolution of honeybee dance dialects. *Proceedings of the Royal Society B* 287:20200190.

Cammarosano M, Weirauch K, Maruhn F, Jendritzki G, **Kohl PL*** (2019). They Wrote on Wax. Wax Boards in the Ancient Near East. *Mesopotamia* LIV (2019):121–180. *contribution to a chapter on honeybees of the Near East and traditional apiculture

Requier F, Garnery L, **Kohl PL**, Njovu HK, Pirk CW, Crewe RM, Steffan-Dewenter I (2019). The conservation of native honey bees is crucial. *Trends in ecology & evolution* 34:789–798.

Kohl PL, Rutschmann B (2018). The neglected bee trees: European beech forests as a home for feral honey bee colonies. *PeerJ* 6:e4602.

Authors' contributions to chapters two, three & four

Chapter two (published):

Kohl, P. L., Rutschmann B., & Steffan-Dewenter I. (2022). Population demography of feral honeybee colonies in central European forests. *Royal Society Open Science* 9:220565

Authors' contribution statement as in publication:

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

Contribution of the candidate in detail:

Publication details	**Description of the own contribution**
Writing of the article Which parts of the article have been written to which extent by the candidate?	PLK wrote all parts of the manuscript and integrated contributions by the co-authors (90%)
Performed research Which experimental procedures have been conducted by the candidate?	Field work make up about 80% and laboratory work made up 20% of the research and PLK performed 50% of the field work plus 100% of the laboratory work making 60% research.
Conceptual design of the research To which extent did the candidate contribute to the conceptional design of the research project?	PLK contributed to the conception of every element of this research (50%)
Data analysis To which extent did the candidate contribute to the data analysis?	PLK performed all data analyses (100%)
Overall contribution of the candidate (in%)	(90%+60%+50%+100%)/4 = 75%

Herewith I confirm that the above description of the specific contributions of the PhD-candidate to the publication is correct,

Name of responsible author Signature Date

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Chapter three (submitted and deposited as a preprint):

Kohl, P. L., D'Alvise, P., Rutschmann, B., Roth, S., Remter, F., Steffan-Dewenter, I. & Hasselmann, M. (2022). Reduced parasite burden in feral honeybee colonies. *bioRxiv* 2022.07.18.500457

Authors' contribution statement as in submitted manuscript:

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.

Contribution of the candidate in detail:

Publication details	**Description of the own contribution**
Writing of the article Which parts of the article have been written to which extent by the candidate?	PLK wrote all parts of the manuscript and integrated contributions by the co-authors (90%)
Performed research Which experimental procedures have been conducted by the candidate?	Field work made up about 50% and laboratory work made up 50% of the research. PLK performed 60% of the field work plus 80% of the laboratory work making 70% research.
Conceptual design of the research To which extent did the candidate contribute to the conceptional design of the research project?	PLK contributed to the conception of every element of this research (80%)
Data analysis To which extent did the candidate contribute to the data analysis?	PLK performed all data analyses (100%)
Overall contribution of the candidate (in%)	(90%+70%+80%+100%)/4 = 85 %

Herewith I confirm that the above description of the specific contributions of the PhD-candidate to the publication is correct,

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Chapter four (submitted):

Kohl, P. L., Rutschmann, B., Sikora, L. G., Wimmer, N., Zahner, V., D'Alvise, P., Hasselmann, M. & Steffan-Dewenter, I. (2023). Parasites, depredators and limited resources as potential drivers of winter mortality of feral honeybee colonies in German forests.

Authors' contribution statement as in submitted manuscript:

P.L.K.: conceptualization, formal analysis, funding acquisition, investigation, methodology, visualization, writing–original draft, writing–review & editing; B.R.: conceptualization, formal analysis, investigation, methodology, visualization, writing–review & editing; P.D.: investigation, methodology, writing–review & editing; L.G.S.: investigation, methodology, writing–review & editing; N.W.: investigation, methodology, writing–review & editing; V.Z.: methodology, resources, writing–review & editing; MH: resources, supervision, writing–review & editing; I.S.D.: supervision, writing–review & editing.

Contribution of the candidate in detail:

Publication details	**Description of the own contribution**
Writing of the article Which parts of the article have been written to which extent by the candidate?	PLK wrote all parts of the manuscript and integrated contributions by the co-authors (90%)
Performed research Which experimental procedures have been conducted by the candidate?	Field work make up about 85% and laboratory work made up about 15% of the research and PLK performed 50% of the field work plus 80% of the laboratory work making about 50% research.
Conceptual design of the research To which extent did the candidate contribute to the conceptional design of the research project?	PLK contributed to the conception of every element of this research (50%)
Data analysis To which extent did the candidate contribute to the data analysis?	PLK contributed 50% to the data analyses
Overall contribution of the candidate (in%)	(90%+50%+50%+50%)/4 = 60%

Herewith I confirm that the above description of the specific contributions of the PhD-candidate to the publication is correct,

Name of responsible author Signature Date

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Affidavit

I hereby declare that my thesis entitled: “The buzz beyond the beehive: population demography, parasite burden and limiting factors of wild-living honeybee colonies in Germany” is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis. Furthermore, I verify that the thesis has not been submitted as part of another examination process neither in identical nor in similar form. Besides I declare that if I do not hold the copyright for figures and paragraphs, I obtained it from the rights holder and that paragraphs and figures have been marked according to law or for figures taken from the internet the hyperlink has been added accordingly.

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Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation: „Das Summen fern des Bienenstocks: Populationsdemographie, Parasitenlast und limitierende Faktoren wildlebender Honigbienenvölker in Deutschland“, eigenständig, d. h. insbesondere selbständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen, als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben. Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat. Weiterhin erkläre ich, dass bei allen Abbildungen und Texten bei denen die Verwertungsrechte (Copyright) nicht bei mir liegen, diese von den Rechtsinhabern eingeholt wurden und die Textstellen bzw. Abbildungen entsprechend den rechtlichen Vorgaben gekennzeichnet sind sowie bei Abbildungen, die dem Internet entnommen wurden, der entsprechende Hypertextlink angegeben wurde.

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